AEIC Fall Meeting 2002 Minutes
October 3-4, 2002
Minneapolis, Minnesota
P.L. Hunst, AEIC Secretary

The AEIC Fall Meeting 2002 was held in Minneapolis, MN at the General Mills site and was co-sponsored by Medallion Labs (Anne Bridges) and Cargill (Randy Giroux). Jim Stave (AEIC President) opened the meeting on October 3 by giving a brief overview of AEIC's history and how the organization has evolved from focusing on immunoassays for chemicals to detection methods for biotechnology products. The core focus of AEIC has always been analytical methodology, particularly validation of methods. The Fall Meeting focused on analytical methodology for food safety.

AEIC Business Meeting:

1) A motion was made and seconded to approve the secretary's minutes of the Spring 2002 meeting.

2) Dean Layton presented a summary of the state of the treasury. The current account balance is approximately $29130. Outstanding dues for 2002 are $1650 which would give a potential new balance of $31180. Total expenses are projected to be $15243 which would leave a projected balance of $13887. A motion was made and seconded to approve the treasurer's report.

Membership: There are currently 7 outstanding membership renewals. A motion was made and seconded to remove all of these from the membership list, except Beacon Analytical. Beacon has been a member for years and it is believed that they will continue. A motion was also made and seconded that the fee for affiliate members should be dropped.

Crediting Bayer: Bayer CropScience ended up paying the hotel bill for the 2002 Spring Meeting in Raleigh, NC which came to ~$3500. A motion was made and seconded to grant Bayer CropScience 7 years of membership for this.

3) *EPA Workshop (D. Grothaus):* The workshop was organized through Mark Ryndal (EPA, Ft. Meade, MD). His lab will be physically validating the methods submitted for biotech products. The workshop will cover PCR and protein detection methods and will be co-sponsored by AACC and AEIC. The workshop will occur in late October, 2002 and essentially EPA will be paying for it. AEIC members requested that EPA be queried during the workshop about electronic recordkeeping, i.e., members want clarification on how this will work.

4) *DNA Paper (A. DeLisle):* An outline of the paper was presented to the membership. Anne Bridges offered that the paper be published in a special section of JAOAC that she has been asked to be editor on. The tentative publication date is early spring 2003. The membership agreed that this would be a good option for publication and the paper

subgroup will try to meet this deadline for publication. The completed paper will also eventually be posted on the AEIC website.

5) *Protein Paper (D. Grothaus):* Cindy Lipton had been hired to coordinate the writing of the paper, however, due to a change in jobs, Cindy has moved on. A request was made to the membership to offer names of suggested consultants who might like to take on the coordination role.

6) *Sampling Paper (S. Charlton):* The paper has taken a "back burner" to the PCR paper. A first draft had been written, however, it will need to be torn apart and reconstructed with a new perspective. Again, Anne offered that it could be published in the special section. Stacy will try to reassemble the original focus group (Stacy, Markus, Ray, Randy, Jim and Paul [Medallion Labs]) to try to meet the publication deadline.

7) *PowerPoint Presentation (D. Layton):* Medallion Labs has taken over the coordination of the slides and has asked that technical reviewers from the membership be assigned to review what has been done. The technical review group volunteers are Frank K. (Neogen), Markus L. (Monsanto), Kalyn B.-D. (Midwest Seeds), Dave G. (Pioneer) and Tom C. (Bayer). Medallion will put together an estimate of the cost to finish the job, i.e., putting into a format for the website.

8) *AEIC Board Nominations (S. Charlton):* Nominations are needed for President, Vice President and Treasurer. It was suggested that the Secretary send out a nomination notice via e-mail to the membership and ask for a return of no later than October 31. Several nominations were made from the floor: President—Stacy Charlton (Syngenta); Vice President—Markus Lipp (Monsanto); Treasurer—Dean Layton (Envirologix). All persons nominated from the floor accepted the nomination. Voting will again be done electronically and will occur between November 1 – 25.

9) *2003 Spring Meeting Agenda/Location:* Agilent (Wilmington, DE) volunteered to host the meeting. Neogen (East Lansing, MI) volunteered to possibly host the 2003 Fall Meeting. The Board will work with Agilent to set dates for the meeting. Possible talks for the meeting include: testing for mycotoxins; AOAC research institute (Markus); DNA detection based on nanospheres (Markus); reference materials for GM products (Randy – Canadian NRC; Don Kendall – NIST; JRC – Markus); identity preservation (Frank S.); assay methods for single amino acid protein changes (Jim S.) [NOTE: the persons in ( ) have agreed to contact for speakers].

ISTA Proficiency Program (S. Ednie, CFIA on behalf of ISTA): ISTA is the International Seed Testing Association which was formed in 1924 to facilitate the international trading of seed. The original goal of the organization was uniformity in seed testing through the use of prescribed methods for seed lot quality estimation (mechanical purity, germinability, varietal purity). It was conformity testing, not compliance testing. ISTA currently has 158 member labs: 80 accredited labs; 70 member countries; 11 private labs. As with most international organizations, there is one

vote per country. ISTA sets the international rules for testing, i.e., sampling, purity, germination, seed health, variety verification, moisture, etc. The rules apply for seeds of agricultural crops and vegetables, flowers, spices, herbs, medicinal plants, trees and shrubs. ISTA has established a referee testing program and has had a proficiency testing program since 1930, basically covering purity and germination. In 2001, ISTA set standards for performance. In 1995, a lab accreditation program was established. To be accredited, a lab must participate in the referee program. ISTA has set forth their vision, strategy, and action for GMO testing which are defined in a paper published in November, 2001 and can be accessed at www.seedtest.org. The paper deals with adventitious presence issue, however, it does not deal with the reverse situation, i.e., GM variety contaminated with non-GM. The ISTA Task Force for GMOs is composed of members from the EU and North America. The US members include Doris Dixon (Monsanto), Tim Gutormson (Midwest Seeds) and R. Payne (USDA). The focus of the task force is uniformity in GMO testing result through a performance-based approach. The task force is active in three directions: 1) writing of an ISTA rules chapter for detection, identification and quantitation of GM seeds in conventional seed lots; 2) organizing proficiency tests on detection and quantitation; and 3) providing technical assistance for member labs. ISTA has conducted a proficiency test for GM corn and the provisional report on these results was issued on September 23, 2002.

Development and Validation of Food Pathogen Assays (M. Mozola, Neogen): Food pathogen assays have only been developed since the late 1980's. Categories of food assays include modified culture (biochemical, filtration methods), immunoassays, conductance/impedance (not too popular) and DNA hybridization (PCR). The interest in the methods is to improve time-to-results vs. culture methods; improving sensitivity and specificity; obtaining objective results and requiring less microbiological expertise. The decision of which method to use is based on the method performance, matrix applicability, cost of reagents, ease of use and labor requirements, training requirements, time-to-results (biggest driver in market) and service from vendor. The steps in assay development include: reagents/assay design, assay optimization, inclusivity/exclusivity testing (separating target and nontarget organisms), pilot food trials, validation, stability testing, field trials and product launch. For assay validation, the following parameters need to be considered: 1) matrices (number and type); 2) number of samples (50 per food type); 3) inoculation protocols (need to used stressed cells); 4) enrichment protocols; 5) protocols for confirmation; and 6) parallel reference methods. Method performance data have not traditionally been published. In those studies that have been published, there have been few comparisons between rapid methods vs. reference methods; use of unstressed cells as inocula; and wrong or inconsistent statistics analyses. Currently, the time-to-results of pathogen methods is:

1) conventional culture	$\geq$ 4 days
2) immunoassay	24-48 hours
3) DNA methods	24-48 hours

PCR Validation (M. Lipp, Monsanto): For PCR validation, most of the pressure comes from food labeling. There are no thresholds on seeds, grains or commodities. The food

thresholds are mostly defined on a weight ratio only but what is actually measured in food is protein concentration and/or gene copy numbers. Validation parameters are well known from analytical chemistry and can be applied for PCR. In-house parameters include precision, bias, LOD/LOQ, selectivity and sensitivity. Collaborative trials are performed to test reproducibility. IUPAC has put forth definitions for collaborative trials. Method performance is defined as the determination of the bias and precision of the analytical method. Material performance study assigns a value and an uncertainty to a characteristic of the material. A lab performance study permits the evaluation of each participant against preset criteria or criteria estimated from the study itself. Other parameters for validation that should be considered include recovery, linearity of the standard curve, fitness-for-purpose, and determination of false negatives/false positives for a qualitative method. There are uncertainties surrounding the extraction of DNA caused by degradation of the DNA itself and impurities from the extraction method. Uncertainties in DNA quantification occur because of single-stranded vs. double stranded distinguishing in UV spectroscopy, size dependence efficiency of PCR and degradation in densitometrical determiantion (different size fragments). The biggest issue for PCR methods is what is actually measured (% DNA) and how to translate that to what is required by the regulations (weight %). Validation is indispensable in characterizing any analytical method but it is important to realize and state the correct units.

DNA-Based Assays for Food Pathogens (P. Mrozinski, Qualicon): *Salmonella*-caused illness causes $1 billion in health costs, lost wages, etc. every year. The leading causes of food borne illness, which are not regulated, include *Campylobacter* and the Norwalk virus. There are no good detection methods for either one. The fundamental question that must be answered: Is the pathogen present? This is done by pathogen screening methods. Some enrichment step is still needed for all screening to get the target organism to a level of detection for ELISA and/or PCR. Accuracy, speed and cost are the major drivers for food pathogens assays. The BAX system was described as an example of a pathogen screening assay.

Emerging Issues in Microbial Food Safety (K. Swanson, General Mills): Food testing is not food safety management. Food must be managed to minimize the risk of exposure. Food safety objectives are linked to public health goals. The President's Council on Food Safety has called for a reduction of 25-50% in annual cases of *Campylobacter, E. coli* O157, *Listeria, Salmonella enteritidis* in eggs. Zero is not an achievable goal. To determine the maximum frequency or concentration of microbial hazard in food at time of consumption that provides the appropriate level of protection, a dose response curve is needed to determine where the food processor should operate. Quantification is needed for this. The basic control strategies of the food processor are 1) controlling the initial levels coming into the plant (purchase specifications, environmental monitoring, good hygienic practices, cleaning, sanitation); 2) controlling the increase of the hazard (time/temperature control, pH, water activity, preservatives); and 3) reducing the hazard (cooking steps, irradiation, acidification). The performance criteria are determined to achieve the outcome required to achieve a food safety objective. Testing may play a role for evaluating the potential for growth and the extent of reduction. Process control is necessary to understand what is going on. More variability in a process

points to an uncontrolled process. Quantification is needed for determining process control.

UPDATES

AACC Check Sample Program: There are currently 587 subscribers. There are 25 check sample services offered. AACC provides statistical evaluation of results comparing data of each lab with all other labs participating in a particular check sample service. Subscribers receive periodic quantitative estimates of their accuracy. The standards of accuracy/precision are determined by the spread of the actual analytical results rather than by an arbitrary decision. The biotech check sample service has a series each for corn and soybeans. Each participant receives 6 rounds of samples/year. The samples are ground, blended and packed and then sent to the participants. For corn, the samples are a combination of Mon810, T25 and Cry9C. For soybean, the samples are Roundup Ready. Subscribers are free to use any analytical method—protein or DNA. Currently, there are 15 subscribers for corn and 15 subscribers for soybean.

GIPSA Verification Program (D. Kendall): There are two programs—biotech and mycotoxins. The mycotoxin check sample program is currently only for the USDA. The mycotoxin kit test program has official certification criteria. For USDA, only certified kits can be used. A fee was established as of August, 2002 for certifying kits: $55/hour which works out to about $5000-7000 per test kit. For the biotech program, there is a verification program for tests of corn and soybeans. The fee is the same as for mycotoxin tests. The proficiency program is currently voluntary and has no fee associated with it. There is a quarterly dissemination of samples. The corn events in the samples include T25, CBH351, Mon810, GA21, E176, BT11, NK603 and for soybean, it is CP4 EPSP. Qualitative results only are reported, however, qualitative and quantitative results will be reported by February, 2003. Participants can use either protein or DNA tests. Currently, there are 17 participants from the US and 16 from outside the US. There has been reasonably good performance by the participants. USDA has also been collaborating with NIST on PCR. Particle size of samples, as it affects DNA extraction efficiency, has been looked at. As predicted, large particles result in a nonhomogeneous mix which results in less DNA extraction and therefore, less DNA detection. They have also looked at commercially available DNA extraction kits and have found that there is clearly operator influence.

TAG (G. Clapper): AOCS has a proficiency program which has participants from 40 nations and over 500 chemists. The intent is to achieve and maintain peak performance of labs. AOCS also has an approved chemist program which recognizes the highly skilled participant. The certified lab program is a dedicated program and allows recognition to superior labs. AOCS is involved with ISO TC34 which has working groups involved with GMO methods, HAACP, and traceability.

ISO Update (R. Giroux): At the Bangkok meeting, the documents on sampling, PCR and the general document were presented. All comments on the PCR and general documents were accepted. The sampling was not approved because the committee indicated they

had not read the guidance documents attached to the document. A subcommittee for sampling was developed for ISO which consisted of delegates from the US, Korea, Thailand, Japan and France. Consensus was reached on the comments, however, all countries, except France, approved the appendices. The appendix discussions will continue at AACC this year. The comments on all the documents were taken to CEN, however, no consensus was reached on the sampling document.

ILSI Training Program in Brazil/Argentina (D. Grothaus): The training program was held in late September, 2002 in Brazil and Argentina. Quantitative and qualitative protein detection methods and qualitative PCR method were taught. The program was well attended and received.

OSTP Adventitious Presence Policy (S. Charlton): The policy proposes actions for EPA, FDA and USDA for earlier involvement in the assessment of biotech plants. The document is really intended for FDA's increased involvement and outlines the proposed approach. At some point during field testing, companies will submit safety data to FDA. FDA will review and make a decision on the safety. If the protein then shows up in the food supply, there will be no recalls since the FDA will have already approved. The document outlines exemption categories for proteins already approved. The food, commodity and technology providers are in favor of the procedure and would like to see it as a mandatory process. OSTP did not recommend making it mandatory. The Federal Register Notices was published on August 2, 2002.