

2007 AEIC Fall Meeting Minutes

P.L. Hunst, AEIC Secretary

The 2007 AEIC Fall Meeting was held in Portland, OR on Oct. 3-4 and was hosted by OMIC USA, an AEIC Member Company. Kazumi Haraguchi, President of OMIC USA, welcomed the group to Portland. There were ~30 attendees at the meeting.

AEIC Business Meeting:

Secretary's Minutes of 2007 Spring Meeting: A motion was made, seconded and favorably voted to approve the minutes of the 2007 Spring Meeting.

Treasurer's Report (D. Layton):

Beginning Balance	\$13,667
2007 Dues	7350

Expenditures (to date)

1 '	
Paper	\$4000
Wire Transfer	11
DE Franchise	25
ANSI/ISO	2925
Board Meeting	0
2007 Spring Meeting	30
Website	168
Bank Service Fee	
Brochure	818
Reprints	
Subscriptions	
Miscellaneous	
Total Expenditures (to date)	7977

Actual Balance	\$13010
Certif. of Deposit	10000
Interest	600
TOTAL BALANCE	\$23614

Membership Update (D. Layton):

Large Companies 14 2 unpaid

Small Companies 11 1 unpaid

Associate Members 2 Individual Members 2

Website Update (D. Layton/P. Hunst):

A new webmaster, Mandy Stockstad, has been contracted to update and maintain the AEIC website (www.aeicbiotech.org). She began about a month ago and during that time has worked with the AEIC Secretary to update the current website and begin the redesign. Five designs were presented to the membership and design 1 was chosen with some modifications to the header picture.

AEIC Brochure (D. Layton):

Dean passed out copies of the new AEIC brochure. The brochure is intended as a "leave behind" at meetings, workshops, conferences, etc. to provide awareness of AEIC and its mission/objectives. The brochure includes information on meetings, accomplishments, publications, and how to join the consortium. Five hundred copies were initially printed and it was suggested that an additional 1000 copies be printed. It was also suggested that the re-designed logo be trademarked at the next printing.

AEIC Board Nominations (D. Dixon):

The President and Vice President Board positions were opened for nomination. The following nominations were accepted at the meeting:

President: Gina Clapper (AOCS)

Vice President: Mike Thompson (BioDiagnostics)

Additional nominations were solicited from the membership via e-mail by Oct. 17. Voting for the Board positions will occur in November via e-mail.

Updates:

NIST: Ray Shillito informed the Membership that Marcia Holden is looking at 35S NOS screening methods. She is conducting a survey of how many 35 S NOS methods there are and will then compare the methods and the reference materials.

CODEX Taskforce on Biotechnology: Ray Shillito reported that the TF is adding an Annex to the current document on AP. Detection methods are no longer required in the database; however, the EU will post a link to the JRC website with the methods. Gina Clapper will circulate the document for comment.

GMO Detection Methods Conference, Como, Italy: AEIC Members will submit two abstracts and possibly a third:

PCR paper abstract: F.Spiegelhalter/R. Shillito

Protein paper abstract: D. Grothaus

Possible third abstract on mini-collab between D.Layton and F.Spiegelhalter It is important that AEIC try to bring sound, practical science to the meeting. Also, there was some discussion regarding an AEIC administered short course/training/satellite meeting [to increase AEIC exposure] before the conference.

ISO TC34 WG7: Gina Clapper reported that the WG7 meeting for late November in Japan had been postponed. She requested volunteers to work on the Protein based methods document revision and for people to start thinking about implementing the TS to the Annexes of the current documents. The new SC for Biomarkers is out for vote with a closing date in December 2007.

AEIC Spring Meeting 2008: Eurofins GeneScan volunteered to host the Spring Meeting in New Orleans. Membership preferred the French Quarter for location instead of Metairie (where the laboratory is located). Possible suggested topics included:

- Sampling Issues; presentation by Russell Marine Group;
- Invite Marcia Holden to update the Membership on her screening method work;
- Uncertainty Measurements;
- New SC information (provided the vote is positive);
- some sort of focus on Asia/Mexico/Caribbean for import/export (Satoshi Futo mentioned at AACC that he would be interested in attending/presenting).

The Fall meeting will be hosted by BioDiagnostics in Minneapolis. The focus will be canola.

Other Business: Ray Shillito mentioned training opportunities in the Pacific Rim for February 2008 possibly in Singapore and then move the workshop to India. He will also be in Vietnam this December to attend a meeting of regulators.

Invited Talks:

Wheat Quality 101 (Gary Hou, Wheat Marketing Center):

The Wheat Marketing Center is a nonprofit organization representing U.S. wheat farmers. It is supported by wheat commissions in seven states.

Wheat is divided in to classes:

- hard red winter (majority of wheat grown in the U.S.)
- hard red spring (grown primarily in the Dakotas and Minnesota)
- soft red winter (MO, IL, IN, OH and other Eastern states)
- durum (ND, MT)
- hard white (ND)
- soft white (WA, OR, ID)

Color of the kernels is the obvious difference between the classes. However, they also differ by protein, gluten, etc. Hard red spring wheat has high protein, strong gluten and

high water absorption. Hard red winter has 11-13% protein, mellow gluten and medium hardness. Hard white has 12-14% protein, mellow gluten, and white bran. Soft red winter has 9-10% protein, weak gluten and a soft endosperm. Soft white has 9-10% protein, weak gluten, soft endosperm and white bran. Durum has 13-15% protein, hard gluten and white bran.

Wheat is used in a variety of applications which include breads, noodles, flour, Asian noodles, crackers, pie crust, doughnuts, cookies and cakes. Wheat quality has a different meaning to different groups of people. To wheat breeders, quality is disease/drought resistance. To wheat growers, it is the yield. To exporters, quality allows them to sell as much as possible. To wheat millers, kernel size is important since it yields more flour and lower ash for quality flour. To end-users such as noodle makers, quality is defined by the finished product quality. To consumers, quality is the taste/texture of the products they buy.

General varietal qualities include disease resistance, parents for superior end-use quality and uniform performance and stability. Specific traits include breeding for gluten strength for the hard wheat class.

Wheat flour testing includes testing for moisture, ash, protein, falling number, flour color, wet gluten content (glutomatic), dough mixing properties (farinograph), visco-elasticity, dough strength, flour starch properties, texture and color of product (noodles). The falling number measures the viscosity via the effects of sprout damage of the seeds. Sample is placed into water and boiled. If the seeds have sprouted, the paste created from boiling will be thin. The farinograph is used to measure dough mixing properties by measuring the amount of water needed to make a good dough. A mixing curve is created which indicates if a flour contains strong gluten or weak gluten. The extensigraph measures the dough extensibility and resistance to extension. A weak gluten flour will have much lower resistance than a strong gluten flour. The alveograph measures dough strength by inflating the dough into a bubble and then measuring the bubble. A weak gluten flour requires less force to create a bubble than a strong gluten flour. The amylograph measures flour starch properties which is important for noodle properties. If the wheat had sprouted prior to being made into flour, the flour will have low viscosity which is not desirable. The texture analyzer measures the texture of cooked noodles which can be related to sensory evaluation by humans.

In wheat trading, the contracts dictate the specifications. Tests are standardized internationally. Time is money, therefore, tests cannot take long due to the loading of ships. For sampling, a composite sample is taken of every 1000 tons. The composites are pooled and then reduced in size. Vomitoxin testing is done at this time.

There are hundreds to thousands of wheat varieties in the U.S. due to each breeder having the freedom to release their own variety by state. Varieties can be identified by electrophoresis and HPLC. The designation of winter or spring wheat pertains to the planting date. Winter wheat is planted in the fall and harvested in the spring whereas spring wheat is planted in April/May and harvested in the fall.

Rice Seed Quality Control and Sampling Issues (Vikash Anand, California Agri):

California Agri has done all inspections in California since 2005. World rice production was approximately 420 MMT in 2007. Of this, 1.4% was produced in the U.S. Most rice is consumed in the countries where it is produced, resulting in about 30 MMT being exported worldwide. The U.S. exports about one-half of the total it produces. Long grain rice is predominantly planted in the U.S. (AR, CA, LA, MS, MO, TX). California plant more medium/short grain rice than other states. California does not export paddy rice since it would take work away from the mills. Therefore, it deals mostly in finished product. All testing is done under contract and the EU is very concerned about GM rice coming as imports. Sampling for testing is performed via USDA GIPSA specifications and sampling devices used include the Ellis cup, diverter mechanical sampler and the deep bin cup. In farm bins, probe samples are taken and bins are turned to obtain the samples. Trucks are sampled at mills. California Agri will analyze submitted samples for rice, however, for other grains, trucks come right from the field to the testing facilities. Mills do online sampling of the finished product to test for weight and grading.

In California, the organic rice farms are very small. The rice must be produced in certified facilities and the fields must be separated from conventional rice by distance and a buffer zone. All equipment such as driers must be certified. Rice must be able to be traced back to the field from which it came via a paper trail.

International Sampling Forum (Gina Clapper, AOCS/TC34):

The forum organizing committee has suggested the participation of FOSFA, GAFTA, CODEX, AACCI, AOCS, ICC and possibly ISTA as well as representatives from TC34 (SC2 oilseeds, SC4 cereal grains, SC10 animal feeds), surveyors and assessors and governments (USDA GIPSA, Canadian Grain Commission). The timeline for the forum includes an electronic meeting to be held in Dec07 followed by a physical meeting the afternoon of March 8, 2008 in Budapest. Other possible meetings are: May 14-16, 2008 in Seattle at the AOCS Meeting; mid-June, 2008, in Vancouver at the ASTM International Meeting; September, 2008 in Honolulu at the AACCI Meeting or October, 2008 in Paris at the TC34 Plenary Meeting. The convenor's suggestion to hold the meeting prior to the AOCS meeting in May, 2008 for several reasons: Seattle is a port city; it is an easily accessible city; the sooner the better for the meeting; and could also include the Measurement Uncertainty Seminar as a AOCS short course. The proposed schedule is:

May 14>>

- --Start the meeting in the afternoon
- --Introductory presentations
- -- Measurement Uncertainty Seminar
- --Evening reception

May 15>>

- --Port tour
- --Sampling discussions

-- Casual event (billiards, darts, bowling, etc.)

May 16>>

- --Sampling summation
- --White paper group assembled with timeline
- --Adjourn in the afternoon
- --TC34 would meet in the afternoon
- --AOCS Meeting begins May 18

Varietal Identification (Deepak Srivastava, USDA AMS):

Ugly ripe tomatoes are grown in Florida and Mexico and are an heirloom variety with a home-grown flavor. However, ugly ripe tomatoes cannot be shipped out of Florida since the Florida Tomato Commission has insisted that the ugly rip must fall under the marketing order and categorizes the tomato as a "misshapen Florida round". Cherry, roma and greenhouse beefsteak tomatoes are exempt from the marketing order because they are not bred to be "round". In order to amend the Ag Adjustment Act, a method had to be developed to identify the ugly ripe tomatoes to apply for the exemption. Therefore, gene-mapping was employed.

Tomato has the smallest diploid genome among the Solanaceae family (12 pairs of chromosomes). It consists of 950 bp, of which 75% are non-coding regions. Microsatellites are polymorphic loci present in the nuclear DNA that consist of repeating units of 1-4 bp in length. They are present at high levels of inter- and intra-specific crosses and can be used as molecular markers. They are a source of genetic variation and regulate gene expression and protein function. They are found in non-coding DNA. Their numbers can vary between species, varieties and individuals. The sequences flanking the repeats are highly conserved.

To analyze the microsatellites of tomato, forward and reverse primers were used. PCR amplified fragments were analyzed and different varieties were found to have different patterns and numbers. Full-length DNA was obtained from tomato slices by freezing the slices in liquid nitrogen and then grinding. The DNA was isolated by using the Qiagen assay or CTAB. For PCR, the fragments were amplified with a 5' flurorescent tag. Results were obtained by the use of capillary electrophoresis. Alleles appeared as sets of peaks and the number of peaks was dependent on the size of the repeat. The ugly ripe could be distinguished by using a panel with 12 microsatellite loci for tomatoes. This panel was used to differentiate 32 tomato varieties, thus inspectors could distinguish the ugly ripe from other tomatoes and thus, the agricultural act could be amended to exclude the ugly ripe from being classified purely on shape. The use of microsatellite loci can be extended to other crops such as peaches, apple, nectarine, cherries, almonds, apricots, grapes and cashews.

TLDA Measurement Technology (Jon Sherlock, Applied BioSystems):

Nestle in the UK has been working with Applied BioSystems to use this technology to screen for GMO presence in their food products and ingredients.

The TaqMan Low Density Array (TLDA) is a customizable 384-well micro fluidic card, designed for use with the Applied Biosystems 7900HT Fast Real-Time PCR System. It can perform hundreds of real-time PCR reactions simultaneously. TLDAs require minimal amounts of sample, allow for 1 to 8 samples to be run in parallel against 12 to 384 targets. Liquid-handling robotics or complex pipetting to load the samples is not required.

The TLDA Gene Signature Panels are pre-designed TaqMan Arrays containing TaqMan Gene Expression Assays matching genes specific to disease target classes or pathways to facilitate drug discovery and disease research. Genes are chosen from pathway analysis tools, published articles, and collaborators' input.

Applied BioSystems has asked the AEIC Membership if there is an opportunity for this technology to assist seed breeders or testing laboratories.

Jon asked the group to comment on the following list of questions:

- Sensitivity Requirements?
- Cost per sample?
- Speed/Throughput?
- Sample preparation?
- Inhibitors?
- Target # Requirement?

Costs are \$500 per card for customized targets. Fifty to 100 cards would constitute a standardized set and the cost would decrease to \$75 per card. A card with 24 targets would be too much for detecting GMO in food or feed, as most people rely on 35S or nos, but might be practical for molecular breeding purposes. For GMO detection the card would almost need to be rearranged so there is space for 24 or more samples and anywhere from 4 to 8 targets. Sensitivity was a concern, though preamplification is all that is necessary to reach required sensitivities.

The group thanked John and Applied BioSystems for taking the time to present this technology to AEIC.