AEIC Fall Meeting 2016
Meeting Minutes
Oct 4-5, 2016
Decatur, Illinois
Hosted by: EPL Bioanalytical Services

P.L. Hunst, AEIC Secretary

AEIC Business Meeting

The AEIC Business Meeting was called to order by David Levin, President.

The AEIC Group paid tribute to former USDA colleague, Ron Jenkins, who died tragically in a motorcycle accident.

Secretary's Minutes from AEIC Spring Meeting 2016: A motion was made, seconded and voted positive by the membership to approve the minutes as reviewed by the membership.

Treasurer's Report (D. Layton):

Item	Projected	Actual
Beginning as of Jan 1, 2016	**32757**	**32757**
Dues	9500	11000
Interest	250	
TOTAL Income	**9750**	**11050**
Expenditures		
Scientific paper	2000	500
DE Franchise Tax Report	25	25
ANSI/ISO	2900	2900
Board Meeting	700	446
Spring Meeting	4500	12040
Website hosting/maintenance	2000	2034
Credit card processing	596	595
Fall Meeting	4500	*2481
Graphic design (brochures)		
Reprints		
Subscriptions	100	
Misc	100	
TOTAL	**17421**	**21021**
Projected Balance	**25087**	**22788**

*Costs to be updated after Fall Meeting

A motion was made, seconded and voted positive by the membership to approve the Treasurer report.

A discussion was held as to whether the membership dues structure should be changed, i.e., add a new category for very large companies. The AEIC Board will revise the fee structure and bring to the membership for approval at the Spring 2017 meeting.

AEIC Membership Update (D. Layton):

Member Category	Number	Dues	Unpaid Dues
Large	22	$11000	1
Small	14	3500	6
Associate	2	0	
Individual	5	500	3
TOTAL	**43**	**15100**	**10**

Website Maintenance (AEIC Board): There is a need to have a new website company to manage the structure/coding of the website, i.e., ability to address architectural changes and credit card processing. EnviroLogix will inquire of their web host company (in Boston) if they would be interested. The membership was also asked to send suggested names to the Board. The Board will put together what items need to be addressed on the website so that bids can be formulated by interested parties.

Website Content Updating (AEIC Board): A new committee was requested to look at the content of the information on the web pages and to suggest updates/changes. Ray Shillito will chair this effort and be assisted by Matt Cheever, Guomin Shan and Penny Hunst.

ISO Update (R. Shillito): ISO 17025 is the food laboratory standard which people may be familiar with and is required by Codex.

AEIC has been involved since 2000 to offset the EU dominance in proposing/approving standards for 'GMO analysis' and focusing ISO efforts on the technologies rather than the specific use of the technology. ISO committee TC 34/SC 16 is the avenues for AEIC members to participate in ISO standards setting. Standards on PCR, gene chips, varietal identification, and meat speciation are all being developed, as well as a standard on validation of qualitative methods. There is a QA system standarad for plant pathology laboratories in the system. A QA system for biomolecular marker laboratories is also being proposed as well as a sub-sampling method (Japan is drafting).
The next global meeting will be held Sept 4-8, 2017 in Washington DC, and if people are interested, they can participate as an observer or delegate. USDA provides funding of $10,000/year and matching funds come from the private sector. Ray encouraged AEIC members to become more involved both as active participants on the technical committees and monetarily by providing funding.

AEIC Spring Meeting 2017: Thermo-Fisher indicated that a meeting at their Santa Clara, CA site may be a possibility. The Secretary will also check with OMIC USA (Portland, OR) as they had expressed interest in hosting a meeting. Dow also expressed interest in hosting the Fall 2017 meeting.

Suggested topics for the meeting were as follows:

- Evolving technologies: high throughput, upcoming technologies for detection

- How to apply new technology to point of need
- Value capture downstream in the value chain: how to bring new products to market in small crops; Simplot and potatoes; USDA GMO labeling mandate
- Seed testing (reserve for Fall Meeting 2017)

Possible dates for the Spring Meeting would be April 4-5 or April 18-19.

For future meetings, the following suggestions were given:

- Focus on seed testing – suggest Midwest location (Indianapolis)
- San Francisco: algal products (Terra Via, Solozyme)
- St. Louis or Milwaukee: beer brewing cultures
- UC Davis: wine culture
- USDA Idaho or West Virginia: aquaculture/GM salmon

AEIC Goals and Activities (D. Levin): The membership made the following suggestions:

- GMO labeling: low level detection; detection in the food chain; synopsis of key points of application of new technologies; technologies for output traits
- Guidance document on tolerances and how to meet for processed products (T. Geng volunteered to put together a proposal for the paper)
- Gene editing task force: Canada will regulate if trait is novel; can SNPs bet detected in bulk seed and is it validated? (Ryan J., Denise and Clara will put together a proposal)
- ILSI Task Force replacement: table until Spring Meeting

Composition Working Group Update (C. Maxwell): The following are the topics the working group is undertaking:

- Cyclpropenoic acid methods: methods to keep molecules intact
- The ELLA test is now at AOCS.
- EPL has been working on multiplex methods for fat soluble vitamins.
- Endogenous allergen detection and quantitation: ELISA, mass spectroscopy, others?
- Harmonizing methods for proximate analyses.
- Trypsin inhibitor in corn: artifact or real?
- Working group will discuss whether AOCS will take methods to Codex.
- Working group will discuss costs of meetings and how these are funded by AEIC.

AEIC Election: Nominations for Vice President: The Vice President of AEIC is a 3 year commitment by an individual: year 1>VP; year 2> President; year 3> Past President. The duties of the VP include attending/participating in the AEIC Board meeting to plan the year and work with the President and Past President to organize the Spring and Fall meetings.

The following people were nominated and accepted the nomination:

- Ray Shillito (Bayer)
- Dave Rambow (Agdia)
- Elisa Leyva-Guerrero (Monsanto)

Nominations will be accepted through Oct 31 and may be sent via email to the Secretary (penny.hunst@bayer.com). Ballots will be emailed out to the membership in early Nov.

Presentations

The AEIC group was welcomed to Decatur by Decatur Economic Development. Decatur was the site of the first commercial soybean crushing plant in the 1920s. The soybean special train made Decatur the soybean capitol of the world. A.E. Staley (Gene) was the initiator of these projects. He also assisted in the founding of the Chicago Bears football team which originally started as a team in Decatur coached by George Hallis.

Decatur is a major contributor to Illinois agriculture as it is the home of Archer Daniels Midland (ADM). Recently, Howard Buffett has donated $6 million for projects in Decatur to contribute to its transformation.

Is Soybean a Big Deal? (M. Winkle, United Soybean Board): Soybean is the second largest commodity crop in the U.S. It is an oilseed and protein crop (19% oil, 36% protein, 13% moisture, 19% insoluble carbohydrate, 9% soluble fiber, 4% ash). Soybean oil is used in human food (12.5 billion pounds) and in industrial uses (6.4 billion pounds). Soybean meal is used for animal feed (90% of meal).

In 2016, 83.7 million acres are planted in the U.S. with an estimated 4.2 billion bushels to be harvested. Soybean production has doubled in ~20 years. Soybeans are predominantly grown in the upper Midwest, along the Mississippi River to the south. The Dakotas are now growing more soybeans.

In 2014-15, 1.87 million bushels were crushed in the U.S. Of this, 1.84 million bushels were exported. For oil, 18.96 billion pounds oil was consumed in U.S. Half of the exported soybeans go to China, EU, Mexico, Japan and Indonesia. Some meal and oil are also exported.

The salad oil market is stable for soybean but for baking, the oil needs to be hydrogenated (transfats). Transfat labeling has caused soybean oil use to go down (loss of 4 billion pounds).

The United Soybean Board (USB) is a check-off established in the 1990 Farm Bill. To fund the check-off, 0.5% of all soybean sales go to the check-off program. The states keep half of this money and the other half goes to the national program for promotion and research. The USB consists of 70 farmer directors. The USB vision is to drive innovation beyond the bushel. Funded research is much more focused than in the past. The priorities are improved oil composition and improved meal composition.

Previously, 50% of soybean oil was hydrogenated (transfats). The biodiesel market took on some of the excess oil from food. The edible soybean oil market share went from 80% in 2004 down to 59% today. Canola oil has taken over. For soybean oil to go back into the food market, high oleic needs to be brought in. Expansion plans are being developed with DuPont and Monsanto.

In soybean meal, the protein content decreases over time. A small decrease impacts the meal market, i.e., up to $4/ton price reduction. The upper Midwestern states (MN, ND, SD, IA) have seen the most reduction in protein. The southeastern U.S. has much higher protein levels as does soy grown in Brazil. Soybean production is moving to upper Midwest (Dakotas) due to economic decision of farmers.

Nutritional bundles are being considered for animals vs. just crude protein. One project is enhanced energy meal: how to move raffinose to sucrose. Another project is reduced carbohydrate soybean: replace by high oil/crude protein.

USB has a focused 5 year plan which is targeting $0.5 billion in a collaborative platform to look at constituent pricing (maximize capture/distribution of value) and focusing on end-user needs (align prices with constituents that are valuable to end-users).
Soybean Breeding (M. Rehman, Dow): Breeding is about genetic gain. Soybean yields have increased from 28.1 bu/ac in 1984 to 48 bu/ac in 2015 which is a 70% improvement in yield. The average genetic gain/year adds 0.5 bu/ac which adds $354 million profit/year.

Genetically-modified (GM) soybeans have been found to reduce pesticide usage by 23 million pounds, reduce production costs of $1.5 billion and increase the farmer profit by 68%. GM soybeans contribute to resource efficiency by decreasing land use, soil erosion, irrigation, energy use and carbon emissions. Soybean research has increased to $4 billion in 2015.

Soybean is sensitive to photoperiod, thus, varieties are classified according to "zones" in the U.S. Zone 0 is in the far north and zone 6 is in southern Georgia. Soy acres are also increasing in western Canada so there is now a need for zone 00 and 000 variety maturity. Brazil has five maturity zones and Argentina has four maturity zones. Soybean cyst nematode (SCN) resistance is a "must" for yield and needs to be incorporated into varieties for each zone. Other important resistance for variety incorporation are sudden death syndrome (2), Sclerotinia (3), and Phytophthora (4).

Germplasm diversity is very narrow in the Americas. North America germplasm is based on a limited set: 10 lines in the north and 7 lines in the south. Landraces have not made significant contributions for yield in North America. Also, the use of *Glycine soja* as a source of yield genes has not been successful. Plant introductions may be used but it takes patience.

Genetic gain is the efficiency of selection over the amount of time required to accomplish. Genetic gain is driven by selection intensity/accuracy, genetic variation and cycle time. For cycle time, there are multiple generations/year. To improve, marker-assisted selection, fast-crossing strategies, genomic selection, single seed descent are all being used/explored for genetic gain. Double haploids have not yet been used in soybean.

The most common soybean breeding is forward breeding. This entails a parental cross and then selfing progeny. The F5 generation is when the usual first year of yield testing is done. Yield testing needs to be done in multiple environments. Back-crossing breeding is also done using a recurrent parent. Marker-assisted selection (MAS) allows the front-load yield testing with the appropriate trait package, i.e., lines without traits are not tested for yield. Predictive or genomic breeding technology improves quantitative traits in large plant populations by using the whole genome molecular markers. It delivers holistic solutions to breeding by learning from the past performance to inform selection of lines. This reduces the cycle time and eliminates low performing genotypes.

For the future, traits for disease resistance, quality, insect and herbicide resistance, drought and flood are needed. For yield increase, more nodes/plant, more pods/node resulting in more seeds.

DuPont Pioneer 2016 Soybean Research Pipeline Overview (M. Dahmer, DuPont Pioneer): DuPont Pioneer is now 90 years old and has been breeding soybean since 1970. The intent is to increase/protect yield, improve input efficiency and increase end user value. DuPont Pioneer has 4000 researchers at 100 sites in 25 countries. Value to soybean is provided by native and genetically modified

(GM) traits. The vision is to deliver the right product for the right field which requires advanced analytics and a reward system for those growers who adopt the technology.

The development pipeline stages are as follows:

- Phase 1: proof of concept
- Phase 2: early development
- Phase 3: advance development>testing in many environments, regulatory testing and dossier development
- Phase 4: pre-launch>regulatory testing, trait introgression, pre-marketing activities
- Phase 5: launch>regulatory approvals, commercial sales, stewardship of products

The pipeline has a range of projects at different phases. North American and Latin America are the focus geographies. For herbicide tolerance, the RR2 Xtend and LibertyLink traits have been introgressed. BOLT technology which is a next generation sulfonyl urea tolerance is also being developed. Traits which increase the end-use value are Plenish (high oleic) and increased oil.

In Latin America, insect resistance traits effective against velvetbean caterpillar, soybean looper, armyworm, corn earworm are being launched. Pioneer is also looking for non-Bt insect actives. Asian soybean rust is also a big problem and new source of resistance has been identified from chickpea.

Plenish soybean has high oleic oil (20% less saturated fat). This trait is still waiting for EU approvals of stacked trait products (Plenish +herbicide tolerance) in order to be launched. A trait for increased oil and meal in Phase 2.

The GM regulatory environment is an evolving environment which has continued complexity, asynchronous approvals, co-existence issues, adventitious presence and low-level presence issues. There is a need for detection analytics other than immunochemistry for new traits in pipeline.

CRISPR-Cas in Corn (C. Alarcon, DuPont Pioneer): There has been a long journey to being able to specifically target changes in a genome. The evolution has included meganucleases, TALENS, zinc fingers and now CRISPR-Cas (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats-**C**RISPR **As**sociated). Zinc finger technology (2001-03) requires weeks to months product development time. Meganucleases (2006-07) requires months development time with variable quality and $$$$$ investment. CRISPR-Cas is a big improvement over all of these and is the fastest moving field in biology. Applications are being developed in agriculture, humans, therapeutics and diseases.

DNA breaks and repairs happen in nature. All organisms have the ability to repair DNA. However, no all repairs are precise such as in non-homologous end joining. Breaks/repairs are random and occur all over the genome. CRISPR-Cas is a break/repair system that is directed and not random. The Cas9 enzyme binds to targeted DNA and makes a break. The guide RNA directs the Cas9 enzyme. The outcomes from the CRISPR-Cas break/repair can be mutagenesis (knockouts, functional analysis), editing (changing gene function), integration (targeted insertion, gene stacking). CRISPR-Cas can also be used as an advanced breeding tool. Conventional breeding requires 5-7 backcrosses to bring a single trait in. Bringing in more traits in a plant is complicated. CRISPR-Cas allows the movement of a trait in a single step and the trait will have 100% of the genetic background.

In order to deploy CRISPR-Cas, there is a need to understand elite genetics, i.e., high quality sequencing of the elite germplasm and informatics tools and infrastructure to analysis and tracking. Also, the

delivery into elite genetics requires a routine transformation of elite germplasm to create elite super donors. Gene editing is needed for superior activity and targeting specificity using in-house and collaborators' expertise.

For stacked trait products, targeted insertions do not disrupt genes and position effects are known or minimized. The breeding process is simplified. A genomic landing pad using a promoter trap allows the stacking of multiple traits.

DuPont Pioneer is using CRISPR-Cas for waxy corn. Waxy corn has a candlewax appearance and contains 97% amylopectin. It has industrial applications and food uses. It is an identity preserved product which is processed into starch and has a limited export and feed uses. The waxy corn trait via CRISPR-Cas has been discussed with the regulatory agencies. Waxy corn has a natural 30bp deletion which results in a 5-10% yield penalty. CRISPR-Cas provided the opportunity to improve production into the newest hybrids. The decision was made not to mimic the 30bp deletion but to remove the gene and make super donors. No DNA from CRISPR or guide RNA is left in the plant. The CRISPR-Cas waxy corn is ready for field advancement trials and it is projected it will be on the market in a total of 5 years. Pioneer has a letter from USDA indicating that it is not a regulated product. FDA is also supportive.

It is a common perception that neither the gene gun nor Agrobacterium can deliver protein into cells. However, pre-assembled CRISPR-Cas9 ribonucleoprotein (RNP) can be delivered biolistically (Nature Biotechnology 33(11), Nov 2015). Thus, plants can be generated without a selectable marker. Editing frequency is comparable to DNA vector delivery and there is no offsite targeting. Delivery of Cas9 complex co-bombarded with single-strand oligomers as a repair template may alleviate regulatory concerns that current GM plants bring up.

Precision Animal Breeding as a Sustainable, Non-GMO Solution for Genetic Improvement (T. Sonstegard, Acceligen-Recombinetics): Science is currently in a genome editing revolution. The human genome project enabled the mapping of other genomes. The use of TALENS and CRISPR-Cas9 has increased so that genome editing is fueling numerous biotech companies.

Recombinetics is a resource company that only works in animal agriculture. Within Recombinetics, Surrogen creates animals for testing human cures; Regenevida grows human parts and DOD funded; and Acceligen improves livestock.

Acceligen improves livestock to have healthy, productive animals that can sustainably feed a growing planet. The focus is on farm productivity, sustainability consumer benefits and animal welfare. Most traits are qualitative as quantitative traits require many variants to add up for the phenotype. The traits are deployed via *in-vitro* fertilization using precise editing by bovine embryo injection. This tens to cause mosaic animals, i.e., not every cell is edited.

Polled (dehorned) dairy and beef cattle: Fifteen million animals are dehorned each year at a cost of $5-20/animal. Calf mortality does occur and results in $138 million loss. Animal welfare is imperative. Hornless genetics have existed in some beef breeds for 1000 years. Traditional breeding of the trait is not practical as the gene frequency is low. Gene editing resolves the issues.

SLICK cattle: The cattle are heat tolerant and maintain a consistent level of feed uptake. There is no nutritional stress and more efficient forage conversion. Thus, cattle carrying this trait will develop more

predictably under high heat conditions and allows the rancher to predict more accurately the time to market.

Double-muscled cattle: These cattle can increase meat yields by 7-30%. It is a naturally-occurring trait which results in the knockout of myastatin. The meat is healthier and smaller inputs needed to raise the cattle promote sustainability. Acceligen is currently applying for permits in Brazil.

All the traits Acceligen is working on occur naturally and fall under non-GMO status. The company is filing for GRAS (Generally Regarded as Safe) status since genes have been in the human diet for years.

Disease resistance traits are being explored for foot and mouth disease, shipping fever, PRRS (porcine reproductive respiratory syndrome) virus and influenza virus. There are no naturally-occurring resistance genes for these.

Acceligen expects the first approvals of the edited traits in 2017. The key to success if picking the right trait(s) and improving deployment of the traits. The National Academy of Sciences affirms that gene editing is a natural extension of selective livestock breeding so this adds to the argument that the traits should be considered as GRAS.

Regulatory Landscape for Products of Genome Editing (T. Munykwa, Syngenta): Science is at the crossroads in the advancement of plant breeding. Future innovations depend on the existence of sound regulatory policies for plant breeding innovations (PBI) such as editing, cisgenesis, intragenesis, transgenesis as breeding tool. PBIs cut DNA and then the DNA repairs. These changes are classified as SDN1 (site-directed nuclease), SDN2 and SDN3. SDN1 and SDN2 result in small changes to the native DNA. SDN3 has a new DNA gene expression cassette which may contain foreign DNA. How are products similar to conventional breeding going to be regulated? Will SDN1 and SDN2 not be regulated since they are similar to conventional mutations? Will SDN3 be subject to undue data requirements?

Countries approach gene edited crops and products based on different criteria. Canada will regulate a product based on whether the trait is novel. The U.S. regulates on whether the trait/product is a plant pest or pesticide. Argentina is implementing a policy which exempts SDN1 and SDN2 products. The EU, South Africa, China, Korea, Japan, Philippines and Australia/New Zealand are having active discusssions on regulations. India, Vietnam, Thailand, Indonesia and Pakistan are currently just watching the developments of the other countries.

The USDA has examples of gene edited products that they have already passed judgment on. This can be found at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated. PBI produced plant varieties similar or indistinguishable from conventionally bred varieties should not be differentially regulated. Genetic variation in the final product would not be covered under GM law if there is no novel combination of genetic material and the final product contains solely stably inherited genetic material. Regulations are needed if the existing paradigms are used or specific risks are focused on or characteristics of products are taken into account.

GMO Perspective from a Farmer's View (D. Brown, Illinois Farmer): Mr. Brown's family has farmed in the Decatur, IL area since 1867. He has adapted the new technologies, such as GM crops, on his farm. Mr. Brown's view is that the technology providers failed to do public perception studies prior to launching the new technologies. This has left the farmers/growers to defend the technology providers since the

farmers are the end users. GM crops protect the farmers and help the growth of larger farms. However, companies such as Monsanto, charge $400/bag of elite genetics and farmers would like to know if Monsanto or others will cover these costs when the farm recession hits. Roundup herbicide is failing quickly and the CRW trait is failing even though the farmer is paying $400/bag. Palmer amaranth is a real problem in fields and glyphosate does not control. Dicamba, an old product, is being resurrected to take care of it but dicamba formulations have drift problems. It is yet to be seen whether the new formulation of dicamba will protect from the drift issues. Other herbicides such as 2,4-D and Liberty (glufosinate) are being used to fill the void. However, the farmers are also culpable in development of increasing weed resistance as they planted 100% herbicide tolerant crops with no refuges. Insect resistant traits now come as "refuge-in-the-bag (RIB)" which helps with assuring a refuge is being planted. Companies still need better public relations for their technologies and farmers need to be responsible. However, companies are no longer farmer partners, i.e., no personal relationships any longer. Pending mergers will take away diversity of choices for farmers. Where is it all going? Farmers could go organic and get more money for the crops but it is difficult to go back in technology as life is easier with the new technologies. Large operations are now moving in to farming and driving down the margins. Farmers could lose $150-200/acre this year. If seed is only for adding to the companies' bottom line profits, farmers will not support.

Characterization of a Transgenic Event by Bioinformatics (P. Song, Dow): A new transgenic event produced via *Agrobacterium*-mediated transformation involves a vector (plasmid, binary plasmid, linear DNA fragment), regulatory elements (promoters, terminators), gene of interest. Transformation may be transient or stable (inserted into a chromosome but the location is not specific). Regulatory agencies ask the following questions:

- Is an endogenous gene or regulatory element disrupted or deleted?
- Does the structure of the transgene remain the same as expected?
- Does the insert of the transgene create a new open reading frame (ORF) that could be associated with an allergen?
- Are there any potential transgenes that could be transferred into microbial genomes (horizontal gene transfer)?

Bioinformatics are used to answer these questions. ORFs consists of segments of codons starting with ATG and ending with TAA, TAG, TGA. A reading frame (RF) is a segment of codons between two stop codons. In the DNA sequence, an ORF and RF may exist in six reading frames.

To characterize an event, a prerequisite is to know the flanking insert sequence and the parental locus in which the transgene was inserted. Several databases can be used such as NCBI for DNA (GenBank) and for protein (SwissProt, etc).

To determine the insert location, the whole insert is sequenced and then compared to the sequence of the gene construct. The endogenous gene/regulatory element or ORF is identified within the parental locus. A BLASTn search can be conducted to see if the endogenous gene is disrupted. The EU EFSA requires a search for stop to stop codons in six frames across the junction between the insert and its flanking borders. A further evaluation is needed if hits are identified. For allergens, a search of >35% similarity over 80 amino acids and a search of eight contiguous amino acids is needed. For toxins, a search of ORFs (30 amino acids) is conducted. EFSA wants the whole insert analyzed for ORFs and searched for potential of horizontal gene transfer to microbial genomes through homologous recombination. The whole insert is searched against bacteria and Archaea genomes. EFSA also requires the re-sequencing of individual events in stacks. SNPs in the gene of interest are looked at to see if an

amino acid change occurred. If so, the bioinformatics then need to be run again against allergen and toxins databases.

Border sequences are unique structure across junction sites which can be used for event specific detection. The plant genome is plastic and biological evolution (i.e., rearrangements) occurs. Events with a negative impact due to the transgene are discarded during the event sorting process and do not reach regulatory agencies. In summary, intensive bioinformatics analyses are applied to the characterization of genetic inserts. Bioinformatics do add some value to safety assessment as any hit from the analyses is subject to further evaluation.

AEIC New Member: Thermo-Fisher (R. Ramadhar, Thermo-Fisher): Thermo-Fisher is an agribusiness and also agrigenomics. It has $17 billion in revenues with $700 million spent on R&D and 50,000 employees in 50 countries. Thermo-Fisher has five premier brands:

- Thermo – instrumentation
- Applied Biosystems
- Invitrogen
- Fisher Scientific – supplier of equipment, reagents, etc.
- Unity Lab Services – service lab equipment

Thermo-Fisher consists of four groups: analytical instruments (19%), life science solutions (25%; includes Affymetrix), specialty diagnostics (19%), lab products and services (37%).

The vision is to accelerate innovation and enhance productivity of customers. The life sciences group includes genetic sciences, biosciences, bio-production and clinical next generation sequencing. For molecular breeding, gene/marker discovery (next gen sequencing), trait mapping and selection (genotyping by sequencing, microarrays), production (qPCR) and regulatory (qPCR) are offered services. Thermo-Fisher also collaborates with customers such as digital PCR for a GM event, genotyping by sequencing, and genome editing of variants.

The Federal Seeds Act and SRTD: Regulatory and Testing Activities (Y. Wu, USDA AMS): SRTD is the Seeds Regulatory Testing Division. The Federal Seeds Lab was established in 1895 and tested seed for mislabeling. The Federal Seeds Act (FSA) was enacted in 1939 to support the 50 states in enforcing their laws. It established the minimum requirements for agricultural and vegetable seed in interstate commerce, i.e., the FSA applies across state lines. It is directed towards seed for seeding purposes and thus flower, tree, shrub or seeds primarily used for re-vegetation are not covered. The purpose of the FSA is promote the accurate labeling of seed thereby protecting seed customers.

The SRTD tests seed samples for purity, germination, noxious weed seeds, pathogens and variety verification. The types of tests include PCR for GMO detection, electrophoresis for variety testing, seed health bioassays and greenhouse grow-outs. Services for NGO entities include:

- No cost quality checks for regulatory test when requested through the state regulatory agencies;
- No cost weed seed identification;
- No cost herbarium samples;
- No/low cost training for seed analysts;
- Training assistance for managing QMS;
- Low cost PVP seed accreditations.

SRTD also provides industry support by participating in:

- AOSA rules committee and ex-officio member of AOSA Executive Board
- ISTA rules committee co-chair and U.S. representative to ISTA
- U.S. OECD seed schemes administrator and U.S. representative to OECD
- Participation on several working groups within UPOV

SRTD publishes a yearly items of interest online journal which features articles related to testing and regulatory issues. It also publishes the state noxious weed seed list and maintains the variety names database.

Any citizen may submit a FSA labeling complaint. There are cooperative agreements between USDA AMS and state departments of agriculture. USDA AMS also has a memorandum of understanding with AOSCA on certification standards and procedures. For more information, consult USDA AMS website (www.ams.usda.gov/isg/seed.htm).

Positive Identification Detection and Diagnostics (K. Smith, Positive ID): The Firefly DX is a handheld PCR device for point of need detection. It evolved from the M-BAND, a freezer-size aerosol sampling device for biological detection of threat agents and the table-top Dragonfly version. The FireflyDX performs PCR and has results in less than 30 minutes. It is run by battery with single button operation and Bluetooth and wifi capabilities for transmitting results. The PCR is real-time TaqMan. Reagents and waste are contained within the cartridge. The battery can run 10-15 cartridges per charge. Cartridges are injection molded and have a shelf-life of 1 year with a cost of $10-50. A RFID chip contains the required protocol on the cartridge. Samples can be withdrawn from the cartridge for sequencing purposes. Positive ID is currently working on plant and seed samples in addition to human samples.

The applications of the Firefly DX include first responders, agriculture (food testing, animal diseases, crop diseases), human infectious diseases, human clinical (non-infectious diseases).

A pilot study has been run testing GM corn and soybean samples containing 0, 0.3, 1.25, 5, 10% GMO. The ABI 7500 was used for benchmark comparison. The CTs were found to line up fairly well with the ABI results. Phase 1 of the study is to develop an internal standard and smooth out the CT curves. Phase 2 will be continued development of the sample preparation cartridge for plant and seed materials. Phase 3 will be the arrangement for field demonstration of the prototype.

DNAble: Update on the State of Art of Isothermal Amplification Technology (D. Shaffer, EnviroLogix):
The challenge for DNAble has quantification for pathogens, GMOs and gene expression. It is difficult to work with plants and seeds. The sample preparation device is coupled with the PCR device which uses a cartridge. TaqGold PCR has a chemically inactive polymerase which is activated after 10 min of heating. The reaction is nick and polymerize chain reaction which involves enzyme activity cycling instead of thermal cycling. The enzymology has been fixed by the engineering of the enzymes.

The fundamentals of the reaction are two primers with engineered tails. The nick and polymerase enzymes are added and a molecular beacon. A reverse transcriptase enzyme is added for RNAble. The DNA is nicked and then the polymerase binds to extend the DNA. This process—cut, extend, displace, repeat—occurs many times. Molecular beacon binds. The “miracle steps” have now been solved by making own enzymes. It is not your average PCR design and is no longer a hunt, peck, hope method.

The new discovery enables a proprietary design algorithm. It is now possible to design a probe to a target and find an assay that works. The assay design automation software is a cloud-based application developed with flexible architecture. The parameters can be user-modified and there now is an additional rule. Thus, the fundamental, probably final layer to the "onion" that is DNAble has been discovered. It is believed that this will usher in a new paradigm for the utility of isothermal amplification.

Protein Multiplexing LC/MS Method Validation (R. Hill, Dow): Protein quantitation is done by monoplex ELISA which is the standard. The challenges to ELISA include the need for mild extraction conditions, volume of data for stacked trait products, antibody cost, time for development, matrix effects and endogenous compounds.

The surrogate peptide LC/MS allows multiplexing and provides qualitative and quantitative results. Extractions can be done under mild or harsh conditions. The challenges are that a peptide is measured versus the intact protein of interest and active versus total protein measurements.

The surrogate peptide LC/MS takes advantage of a unique primary amino acid sequence. The protein is cleaved into smaller pieces using an enzyme which consistently cleaves at specific sites. The peptide fragments resulting from the digestion are quantified. Method validation leveraged established practices from OPPTS 860.1340 and SNACO/825/00 rev.8.1. and the new FDA guidance. Extraction efficiency was performed via serial extraction from tissue expressing the target protein. A minimum of 5 serial extractions were performed and analyzed via LC MS/MS. Digestion efficiency was performed with a microbial protein standard and tissue expressing the protein. The digestion was done on a time course and the reaction was quenched at various time points. Specificity/selectivity was carried out using trypsin to specifically cleave the C terminus of lysine and arginine residues. Signature peptides were confirmed via a BLAST search. Each peptide was 6 to 20 amino acids. Chromatographic separation was carried out by LC MS/MS. Linearity was determined by a peptide standard curve of 5 points. Sensitivity was determined by protein fortification into control tissue at LOQ level over multiple days. Accuracy/precision/ruggedness was done by using microbial protein fortified into control tissue. Six replications of LOD, LOQ, mid-range and ULOQ were used. Stability of the protein extract following digestion was found to be 7 days. Cross-validation was necessary if data to be submitted to a regulatory agency.

A ring trial was carried out to reflect a true external analysis. Six replications of the same CEA sample was supplied to all seven labs in the trial. The trial showed that the method was robust using a very limited sample material. Some of the labs did not have enough material to optimize the method. Results were comparable among the labs.

The surrogate peptide method is suitable to measure co-expressed proteins. Validation mirrors accepted practices.

Archer Daniels Midland (ADM) Overview (M. Matlock, ADM): ADM is a premier player in the global agribusiness. ADM has 32,300 employees in 740 facilities with $67 billion in net sales. It deals in grain origination/transportation, processing into food, feed and industrial uses and value-added ingredients. ADM was founded by Archer and Daniels to process flax seed. The ag services interfaces with farmers globally and is all about moving grains around. ADM has 28,000 rail cars which assist in the movement of grain.

Corn is processed into 30 different products. ADM has 14 plants in 7 countries that deal with corn processing. The grind capacity is 3 million bushels/day. The products include sweeteners, starches (for paper, corrugated cardboard), alcohol (fuel, beverages), amino acids (lysine), bio-based propylene glycol (food/beverages, de-icing agent).

Oilseeds are processed into 20 different products. ADM has a 150,000 tons/day crushing capacity and 120+ production facilities. Oilseeds include soybean, cotton, flax, sunflower, peanut, palm and canola. Identity-preserved soybeans are grown under farmer contracts. The Decatur east plant is used once/year to process these. Costs are passed on to end customers.

There is a need for stable oils for frying. There is excitement about high oleic canola and also high oleic soybean (once all approvals have been obtained). Oils are blended to make transfat-free functional/nutritional solid fats. This product is called NovoLipid and is from a partnership with Novozymes. Stearic acid is more similar to oleic acid but does not raise cholesterol levels. The human body converts stearic acid to oleic acid.

Lecithin from soybean is an agricultural adjuvant. It is used in micro-emulsion formulations that have penetrating, translocating, wetting, acidifying properties. Vegetable proteins are more productive since more protein is produced per acre of land for soybean than milk, eggs, beef. ADM's Dr. Atkinson originally developed TVP (textured vegetable protein) in the 1950s. Puffed soy powder was made into a meat-like substance. In 2017, a new meat analog will be launched. It does not contain any binders and consists of water, soy protein and flavors. ADM acquired Wild Flavors which has the broadest portfolio of on-trend ingredients. The specialty ingredients market is a $50 billion market with $30 billion from ingredients and $20 billion from flavors. The market is growing 5-6%. The acquisition of Wild Flavors brought more local labs to ADM which allows making ingredients for local tastes.

A tour of ADM was held in the afternoon of Oct 5.

Fall Meeting 2016 Attendees:

Name	**Organization**
Angela Umthun	Stine Biotechnology
Ashley Fisher	Simplot
Bernd Schoel	Genetic ID
Billy Hernan	EPL
Brian Beecher	USDA GIPSA
Carl Maxwell	DuPont Pioneer
Charles Pick	seqID
Christie Johnson	EPL
Christine Atkinson	AOCS
Clara Alarcon	DuPont Pioneer
Corinne Wrigley	EPL
Dan Shaffer	EnviroLogix
Dan Smith	Genetic ID
Dave Rambow	Agdia
David Levin	Covance
David Syme	Bayer

Dean Layton	EnviroLogix
Donna Houchins	Romer Labs
Doug Miller	IL Crop Improvement Assn
Dylan Wempen	EPL
Elisa Leyva-Guerrero	Monsanto
Frank Spiegelhalter	Eurofins Genescan
Fred Claussen	EPL
Gina Clapper	Merieux NutriSciences
Guomin Shan	Dow
Jeff Gillikin	NC State Univ.
Jeff Habig	Simplot
Joe Miller	Monsanto
Joe Warnick	EPL
John Lawry	Covance
Joshua Kuipers	Agdia
Kai Liu	Eurofins NAC
Keith Persons	Eurofins Nutrition Analysis Center
Kimothy Smith	PositiveID Corp.
Lance Workman	AOCS
Li Sheng	EPL
Lisa Waddell	SGS
Luke Muschinske	Covance
Maqsood Rehman	Dow
Mark Dahmer	DuPont Pioneer
Mark Winkle	United Soybean Board
Mary Gadola	Neogen
Matt Cheever	Bayer
Michelle Smith	EPL
Nancy Gillikin	Bayer
Nevena Djuranovic	EnviroLogix
Penny Hunst	Bayer
Ping Song	Dow
Ravindra Ramadhar	Thermo Fisher
Ray Shillito	Bayer
Rich Wilson	Oilseeds/Biosciences Consulting
Robin King	EPL
Ryan Hill	Dow
Ryan Johnson	BASF
Sean White	EPL
Tao Geng	Monsanto
Taylor Stadler	Eurofins NAC
Yongcheng Wang	Monsanto

AEIC Composition Working Group
AEIC Fall Meeting – Decatur, IL

10/6/2016

Introductions

Carl Maxwell (DuPont Pioneer), Nancy Gillikin (Bayer), Fred Claussen (EPL), Kai Liu (Eurofins), Lance Workman (AOCS), Christina Johnson (EPL), Tao Geng (Monsanto), Robin King (EPL), Michelle Smith (EPL), Joe Warnick (EPL), Sean White (EPL), Yangcheng Wang (Monsanto), John Lawry (Covance), Keith Persons (Eurofins), Taylor Statdler (Eurofins), Brenda Dickey (EPL), Luke Muschinske (Covance), Elisa Leyva-Guerrero (Monsanto), Dylan Wempen (EPL)

By Phone – Greg Tilton (Monsanto), Karen Launis (Syngenta), Brandon Fast (Dow), Barb Mitchell (Covance)

- Anti-Trust Statement and Mission Statement were read.
- Notes from the Spring Meeting were reviewed.
    - Update on ELLA method, Barb presented on CPFA, Fred discussed the new Fat Soluble Vitamin (FSV) Assay, Tao spoke about allergens, Proximate method review based on method references given by Eurofins and EPL (Update: Nancy had not yet created subgroup for this), Inositol discussions- what is being measured and how to define, Method Harmonization of analyte endpoints (Update: CropLife is taking this over), Free Gossypol discussions- is it a relevant analyte (Update: OECD and EFSA still say to analyze so analysis may continue, no further opinions)

ACTION ITEMS OR DECISIONS ARE IN BOLD BELOW

ELLA Method Status – Lance Workman

- Lance has taken over this project and has put the method in the AOCS format and hopes to get it submitted soon.
- **Elisa is looking it over and will edit as needed.**
- **Tao Geng, EPL, and Covance volunteered to also look it over once Lance makes changes from Elisa and sends to these parties**
- **Reviewers will have a deadline of 2 weeks**
- History- Methods committee held a vote in Fall 2015 but a majority was not held. At that time, it was a rough draft.
- Keith suggested **sending it enough in advance of the next meeting (April 30, 2017) so it could be reviewed prior to the meeting**
- Greg suggested to send around March
- This is already a validated method and CROs have already begun using it
- **Lance will check in with Unified Methods Committee.**

CPFA – Barb Mitchell

- Currently have data from 9 labs after the USDA retested with a more appropriate extraction
- Hopes to get all data to AOCS to run statistics; reformatting may be required.
- **Timeline- Barb will get data to Lance the week of 10/10/2016**. **Lance will run statistics**, should take less than a week. Elisa thought there was a template.
- Lance suggested having people look over the method.

- **EPL, Greg Tilton, and Eurofins will review the method.**

Allergens –Tao Geng

- EFSA is drafting new guidance supplemental to the IR of 2013.
- In April 2016, first version was released. Companies provided comments. Recently they asked for comments on the second version. EFSA had a meeting in Brussels recently for opinions.
- Plan is to officially publish in April 2017.
- Working group will focus on the third part of this guidance, which is endogenous allergens.
- The guidance states they should be measured compositionally and how many should be measured. This guidance added a couple more than the OECD suggested.
- Compared to the Dow presentation, this document included at least one more allergen.
- The question now is how we deal with this new allergen and which method should be used.
- Tao suggested doing a ring trial and treat it similar to the ELLA and CPFA method and believe that it would be a shorter timeframe to use the LC-MS approach
- CLI sent comments to EFSA but these were more around the number of allergens rather than harmonization of them. Will CLI work on the harmonization of the allergens list?
- Guidance has not indicated whether or not standing studies will require the testing.
- Dow has currently not had troubles submitting LC-MS data. Monsanto has submitted ELISA data with no problem. DuPont Pioneer, Bayer, and most likely Syngenta will submit using LC-MS.
- Discussions about Gly m 7. NC University – wrote paper that EFSA is using as a reference to suggest including Gly m 7. Most represented companies planning to measure Gly m 7.
- In regards to which allergens are being tested, Covance is following the EFSA document and Eurofins is not doing these analyses yet as it has not determined which location.
- Tao suggested a ring trial that includes the allergens that were in Dow's ring trial as well as Gly m 7. Dow's ring trial included Dow and 6 CRO's.
- Issues surrounding peptides – Should everyone use the same peptides? May discuss different peptides based on the ionization of the various Mass Specs. Dow had suggested in its presentation at AEIC that perhaps we may use one peptide as a parent and another as a confirmatory. Either way, there should be some justification as to why a particular peptide was used.
- Should allergens be added to the ILSI Crop Composition database? At this time the ILSI Crop Composition Database Working Group decided against this for several reasons. The methods are too new and it is unknown how comparable they are. Allergens are not nutrients or anti-nutrients so this may not be the best place for them. May revisit this later or create a separate database.
- **Next steps for the working group will be based on EFSA guidance discussions and endpoint harmonization. Perhaps doing a new ring trial to reach the 8-10 labs.**
- Should the working group continue discussing allergens? These discussions may overlap with other organizations. **We may want to reach out to representatives of those groups to see if they would be interested in taking over the discussions on allergens.**

Fat Soluble Vitamins –Fred Claussen

- At the last meeting, Fred presented his work using the EPL vitamin K_1 method. Seeing poor bridging and values much lower than historical values. Excellent precision but matrix suppression.
- Did post extraction spikes to determine the levels of suppression in each analyte (vitamin K_1, beta carotene, alpha, beta, gamma, and delta tocopherols)
- Possible solutions to these issues

1. Isotopically labelled standards – Some are not available and would have to be custom synthesis which is very expensive (minor tocopherols).
2. More sensitive LC-MS systems – Pros: gets better sensitivity and can ease sample clean-up by diluting away the matrix suppression. Cons: transferability and possible cost associated with transferring the method. Are there even enough labs with this high level technology to dedicate time to a ring trial?

- Our goal for changing the method is to determine all analytes with minimal sample clean-up, potential for high-throughput, reduced cost, improved accuracy and precision, and speed.
- **Decision Point for Sponsor Companies:**
    - **We have two paths we can take. Which is preferred? Would isotopically labelled standards be cheaper if sponsors decided alpha tocopherol is is the only tocopherol required for submission? Asking for feedback.**

Future State of Composition Working Group

- Timing of CWG meetings? **When possible, we will meet before the main meeting, but will meet after if needed.** This will depend more on the location of the meeting and the timing of all phone-ins.
- Luke questioned if AEIC moves to once a year meeting, how often should CWG meet? Meet more? Hold teleconferences?
- Nancy suggested **reinstating sub-groups** to help move things along faster.
- Empowering the leaders of those groups to involve the people associated and to meet with Lance rather than involving the entire working group.
- Defined sub-groups at this time and their leader
    - ELLA – Elisa, CPFA – Barb & Keith, FSV – Fred
- **Nancy will coordinate other member's involvement in each sub-group**
- Spring 2017 meeting will be the last meeting for Carl and Nancy as co-chairs of the group
- Future leadership – Suggested **setting up in the same manner as AEIC with a past president and a current president.** Would need to stay in frequent contact with leaders of the sub-groups.
- **Tao suggested a newsletter. Volunteered to help with newsletter. Will send on other quarters without meetings**.
- Who is allowed to attend the CWG meetings? If a company is a member of AEIC, anyone from the company can attend but it would be ideal to have a representative from each company.

Method Review- Proximates

- Previously had collected methods used by EPL and Eurofins in an effort to find preferred methods. Not the intention to confine CRO's.
- **Nancy created the subgroup which will include: Keith (Eurofins), Fred or designee (EPL), Luke (Covance), Brandon (Dow), Carl (DuPont Pioneer). Nancy will follow up with a conference call.**

Inositol/Phytate

- Some on the CropLife composition endpoint harmonization subcommittee have suggested inositol may be dropped. **The AEIC WG discussion is tabled for now.**
- There are currently 2 methods in the ILSI CCDB– free inositol or total inositol
- If the method is not dropped, there needs to be a clear definition moving forward.
- Regulatory guidance from EuropaBio delineates between *myo*-inositol (free) and phytic acid.

- **Nancy will propose changing the inositol analyte name in the ILSI CCDB to two analyte names that differentiate between the two forms.**

Raffinose – Kai Liu

- 2 main methods for raffinose (GC and LC-based).
- Ethanol used to inhibit degradation during extraction.
- Replaced water with 25% ethanol and get 20% increase in soybeans.
- Conclusion: LC method is preferred. Consider adding ethanol for extraction.
- EPL discussed their method of extraction which uses an ethanol:water solution for maize and a methanol:water solution for soy.

- What changes to methods truly make them a new method?
  - Fred believes since many compendial methods were not validated on our matrices, may not be totally fit for purpose. Must adapt/validate them in the lab with the desired matrix. Cite the original reference.
  - Carl stated they ran into issues publishing methods that aren't "novel enough". This is one reason for us to work with AOCS to create official methods.
  - **CWG to determine if this warrants a new raffinose method**

Trypsin Inhibitor Method – Luke Muschinske

- Presented reasons for needing a new method. At this time, do not have a new method.
- The enzymatic method is tedious and lacks sensitivity. Corn also tends to have a background color.
- Goal is to make a more precise method. But first we must figure out what we are actually measuring.
- **Will attempt to have ideas by the next meeting.**