AEIC Spring Meeting 2015

Secretary's Minutes

P.L. Hunst, AEIC Secretary

The AEIC Spring Meeting 2015 was held on April 15-16 in La Jolla, CA and was hosted by Illumina. K. Malloy (Illumina) welcomed the group to Illumina.

AEIC BUSINESS MEETING

Secretary's Minutes (P. Hunst): The Secretary's minutes of the 2014 Fall Meeting were approved by a motion which was seconded and voted positive to approve.

Treasurer's Report (D. Layton): A wrap-up of 2014 was given as follows:

2014	Proposed	Actual	Notes
Starting balance	29006	39006	
Dues	8000	9571	
Interest	190	2	
Other		1076	Payment from CLI
TOTAL	8190	10649	
Expenditures			
Scientific paper	5000	280	
DE Franchise Tax Rpt	25	30	$5 for change of registered agent
ANSI/ISO	2900	2900	
Board Meeting	900	212	
Spring Meeting	2500	675	
Fall Meeting	2500	7476	
Website	3500	3291	
Bank Service charge		4	
Graphic design			
Reprints	800	375	
Subscriptions	100		
Misc	100		
TOTAL	18325	15243	
Projected Balance	28871	34411	

The projected 2015 budget was given as follows:

2015			
Balance	34412	34412	
Dues	800	1500	
Interest	190		
TOTAL	8190	1500	
Scientific paper	2000		
DE Franchise Tax	25		
ANSI/ISO	2900	2900	
Board Meeting	700	475	
Comp WG (*new)			
Spring Meeting	2500	2519	
Website	2500		
Credit Card Processing	525	101	
Fall Meeting	2500		
Reprints			
Subscriptions	100		
Misc	100		
TOTAL	13850	5995	
Proj Balance	28752	29916	

There was a discussion concerning the Composition Working Group (WG) which has been meeting in conjunction with AEIC as to whether AEIC should monetarily support the WG. The membership agreed that the WG's work does fit with AEIC's mission and voted positively to support. A line item was added to the budget. The level of support will be determined when the AEIC Board receives a proposal from the WG detailing the requested monetary support.

A motion was made, seconded and voted positively to accept the proposed 2015 budget.

Membership Update (D. Layton): An overview of the AEIC membership was given:

	Expected	**Unpaid (2014)**	**$ Unpaid (2014)**
Large	18	4	2000
Small	14	4	1000
Individual	2	0	0
Associate	4	1	100

There was a discussion concerning Eurofins acquisition of Biodiagnostics, i.e., do both entities maintain their membership? After membership discussion, it was concluded that since both operate as separate entities, they should both maintain their membership dues.

Website Update (D. Theide): The website has gone through an update over the past year. Recently, credit card functionality was added for membership dues and for pre-payment for the meeting dinner.

Denise will work with the webmaster company to understand how members are to login to pay for their membership renewal. The website text needs updating which will begin during the summer months. A group of members had previously indicated their interest in doing this.

2015 Fall Meeting (D. Levin): Agdia will host the Fall Meeting in South Bend, Indiana, possibly at Notre Dame University. Suggested dates which will be explored are Sep 23-24 or Oct 21-22.

EPL Laboratory has offered to host the 2016 Fall Meeting. David L. will check with Syngenta to see if they are interested in hosting the 2016 Spring Meeting.

There was a short discussion on suggested topics for the 2015 Fall Meeting. These included:

- Crop update: corn
- Suggested tour: seed conditioning facility near Constantine, IN
- Influence on public perception: possibly have a speaker from USDA AC21 to discuss co-existence between biotech and organic crops (Ryan).
- Farmer followers: hear from "Ag more than ever" and the Farmer and Ranch Alliance
- Suggestion to have Vance from Monsanto give a talk on engaging millennials
- Suggestion to have a talk by Charlie Arnot from the Center for Food Integrity
- New Breeding Technologies: what is coming next? Talks from Celectis and/or Recombinectics?
- AEIC intiatives discussion: what will we do and how?
- Composition: evaluating trypsin inhibitor

Protein Paper (D. Layton): A technical editor is in place and is working on the paper to bring all parts together. The next step will be the submission to a journal.

Muliplexing Validation Working Group (G. Shan): Critical Path Services is drafting a paper on ELISA vs LC-MS and what is required. A question was asked as to whether AEIC will be acknowledged and whether the AEIC Board will be able to have a review of the paper prior to submission. It was agreed that this would be done and also that the membership would be informed of WG meetings and the minutes of those meetings. Monsanto is drafting a paper on multiplexing method validation specifically.

ANSI/ISO Update (G. Clapper): U.S. runs the ISO/TC 34/SC 16 for biomarker analysis and AOCS (Gina) is the administrator. Currently, the WG is working on documents for varietal identification, plant pathogen analysis (led by the U.S.), qualitative method validation, guidance on method submission and meat speciation analysis. The 6th Plenary Session will be in Shanghai in 2016.

Composition Working Group (C. Maxwell): The Working Group (WG) is dealing with nutrient composition analysis which is used in safety assessments of products for determining substantial equivalence by comparing the food source to the existing food. The nutritional status of a GM crop is done in the context of comparison with near isogenic control and conventionally-bred crop. What nutrients to measure per crop is based on the OECD Consensus documents for each crop which delineates what constituents to measure and in what (plant tissue, oil or both). The challenges in nutrient composition analysis include a) regulatory requirements are inconsistent among countries and are also growing; b) current methods used are not optimized for purpose; c) methods are not applied consistently; d) there is a lack of an efficient mechanism to drive change and harmonization; and e) can be a barrier to development of novel traits by universities and small companies. The mission of the WG is to collaborate across the agricultural and analytical industries to develop, validate and publish new

analytical methods that improve safety, quality and speed while making compositional studies for agricultural biotech products more cost effective for both private and public innovators.

Enzyme-Linked Lectin Analysis (ELLA)(G. Clapper): Lectin analysis was based on a difficult assay which employed red blood cell hemagglutination and the results were reported in arbitrary hemagglutination units. The ELLA was developed which employs similar technology as ELISA except for lectins. Currently, the ELLA is specific to soybean agglutination (SBA) and yields results expressed in mg SBA protein/g tissue. A manuscript has been submitted to the Journal of AOCS and the group has received reviewers' comments. Once addressed, the paper will then be published. The natural assay variability results are included in the paper. Currently, a ring study is has been conducted with labs from the U.S., China and India. Six samples (with blind duplicates) were measured and the data appears to be good. It is projected that the ELLA will be a published AOCS method by summer.

Cyclopropenoic and Conventional Fatty Acid(CPFA) Analysis by GC (J. Sabbatini): The cyclopropenoic fatty acids (malvalic, sterculic, dihydrosterculic) are anti-nutrients present in cotton. These fatty acids elevate the melting point of fats in animals fed whole cottonseed and can cause egg yolk discoloration in chickens. They are present in low levels in cotton. The first analysis for CPFA was a colorimetric test followed by titration, spectrophotometric, HPLC or GC testing. There are two reference methods listed in the ILSI Crop Composition database for GC and HPLC. Covance was interested in developing a simultaneous method for the CPFA using GC/FID. CPFA have been found to not breakdown on capillary columns and base transesterification does not break the CPFA ring. The GC/FID gave adequate resolution and a LOQ similar to other methods but very small peaks. Reference standards are not available. The pros and cons for the method are:

PROS	CONS
Combines two methods	free fatty acids not quantitated
Derivatization is fast and easy	standards not readily available
Results are comparable	for sterculic/malvalic

The plan is to take this method to an AOAC collaborative study. Sample materials and quotes for reference materials are being collected.

Fat Soluble Vitamins by Multiplexed HPLC/MS (F. Claussen): The objective was to combine analysis of vitamin K, E and beta-carotene into one method with one single extraction and determinative step and to improve accuracy and precision. The benefits would be to have inter-laboratory harmonization and increase laboratory capacity. For tocopherols, the current methods are HPLC/UPLC with UV or fluorescence. For vitamin K, it is a C30 HPLC with post-column zinc reduction and fluorescence. For beta-carotene, the method is C30 HPLC with UV or spectrophometry. The new proposed method is HPLC-MS/MS and electrospray ionization (ESI). Mass spectrometry affords universal detection, is sensitive and selective available technology, dependable and robust and has a reasonable linear range (100X). On the negative side MS is prone to matrix interferences and requires multiple ionization mechanisms for non-polar analytes. Reversed phase chromatography is preferable to normal phase. The C30 requires longer run time and has less resolution of beta-tocopherols and gamma-tocopherols. PFP does not resolve cis- and trans-vitamin K. Hydrolysis of lipids is necessary to quantitative extract fat soluble vitamins. Base hydrolysis procedures are routinely used for vitamin E and beta-carotene, however, this will degrade vitamin K. Lipase digestion is used for vitamin K. The "to do" list includes a) evaluation of atmospheric pressure ionization, b) evaluation of matrix effects for electrospray ionization and atmospheric pressure ionization, c) need stable isotope internal standards, d) improve resolution of

tocopherols, e) spike/recovery determination, f) extraction/SPE optimization and g) LOD/LOQ determination.

Allergen Sub-Team (M. Petty): Allergen assessments are done on introduced proteins (source of protein, bioinformatics, exposure, etc) and on endogenous allergens (comparative assessment between GM and non-GM plants). The drivers of change for these assessments are based on 1) human sera from allergic individuals difficult to obtain and 2) countries have new regulatory expectations such as requiring information on individual allergens and the EU now requiring inclusion of identified allergens in the comparative compositional analysis. The sub-team survey companies to see what methods are used for endogenous allergens. The methods included ELISA, MS, LC-MS/MS, AQUA-MRM. The sub-team had their first call at the end of Feb 2015. They had a general discussion and plotted a path forward. The group generated a spreadsheet to gather data on analytical methods. Harmonization of methods is not practical, however, method results could be compared with round robin of like samples and then share results from non-GM samples. The group held their second call at the end of March 2015 and discussed suggestions to share more generic assay performance. The next questions the sub-team is considering include how methods are developed and validated and what natural variability data has been generated.

Group Discussion of Opportunities for AEIC (All): One suggestion was for AEIC to help make methods more consistent between CROs and other laboratories as well as working out technical issues for allergens, i.e., make sure methods are measuring the same things. Another suggestion was for AEIC to work on method transfer by delineating a process, the parameters to measure and then harmonizing between groups. Another suggestion was that possibly AEIC could help integrate with agencies on interpretation of new gen sequencing data. This may entail working with the CLI molecular characterization team.

The business meeting was then adjourned.

INVITED TALKS

Overview of ILSI (International Life Sciences Institute) and CLI (CropLife International) (G. Shan): CropLife International (CLI) has a global network of regions. The General Assembly consists of the crop protection associations, plant science associations and biotechnology associations. The Board reports to the General Assembly and the Secretariat reports to the Board. Under the Board, the Crop Protection Steering Committee and Plant Biotech Steering Committee (PBSC) reside. Under the PBSC, there are 8 different teams that deal with various issue areas. Four of these project teams are ERA (Environmental Risk Assessment), DMPT (Detection Methods), STPT (Stacked Traits) and FFPT (Food Feed).

The International Life Sciences Institute (ILSI) promotes global partnerships for a healthier world by advancing the understanding of scientific issues relating to nutrition, food safety, toxicology and risk assessment. ILSI is a neutral forum between academia, government and industry for discussing and advancing scientific issues. The ILSI Board oversees the geographic branches, the global branch and the research foundation. Under the global branch, HESI and 13 technical committees reside. The Research Foundation oversees CERA, CSAFF, RSIA, CIMSANS and nutritiona/health promotion.

Allergencity Assessment (R. Herman, Dow AgroSciences): The ILSI PATC (Protein Allergen Technical Committee) has a multi-sector membership focused on scientific issues associated with detection and evaluation of allergencity. The CLI AET membership is industry focused and implements the science

from the PATC. The PATC actively publishes, having had 7 papers published in 2014-15. And the PATC supports and conducts research. Current research projects include the digestibility working group which is working a on a more physiologically-based simulated gastric fluid (SGF) digestion assay. The protein toxins WG is working approaches to identify protein toxins with the focus on the use of bioinformatics. Another project is investigating the use of the GARD assay to identify allergenic potential of purified proteins using dendritic cells. The PATC also recently sponsored a workshop on potential unintended effects which was held in conjunction with OECD meetings in Paris.

Allergen assessment requires a multi-disciplinary expertise. It encompasses looking at endogenous allergen levels, population sensitization, likelihood of harm of new proteins, and utilizes several disciplines including immunology, epidemiology, immunochemistry, protein biochemistry, digestive enzymology, bioinformatics, crop composition and risk assessment.

Some of the current challenges in allergen assessment are the use of bioinformatics, digestion assays and unintended effects. Under Codex, the guidance has been for bioinformatics that a protein is similar to an allergen if >35% amino acid identity over >80 amino acid sliding window matches. Science says the use of FASTA combined with E-value threshold is superior to percent amino acid (current guidance). The suggestion for policy is to supplement the Codex with FASTA/E-value threshold and then filter results based on E-value for biological relevance. Final results are the same except that regulators receive data that conforms to Codex to satisfy regional requests.

For digestion assays, standards needed for validation do not exist. PATC is working to obtain 5 pairs of structurally related proteins to use to compare traditional SGF parameters with SGF conducted at sub-optimum enzyme concentration and pH followed by simulated intestinal fluid (SIF) assay. The 5 pairs of proteins include a) lipid transfer proteins (peach/strawberry), b) tropomysins (shrimp/pork), c) 2S albumins (peanut/green pea), d) parvalbumin (carp/swordfish) and e) collagen (fish/beef).

Cryptic reading frames are looked for across T-DNA inserts/junctions as these are believed to lead to hidden protein production. A reading frame is defined as spanning from stop-to-stop codons with no promoter being required. The odds of randomly creating an open reading frame that expresses unsafe protein are miniscule. Dow published on this in 2011 (Herman, et al. 2011. GM Crops & Food 2: 4-6).

Few proteins are allergens but most allergens are proteins, therefore, evaluating novel food proteins for allergenicity is prudent.

CLI Detection Methods Project Team (R. Guo, Bayer CropScience): The CLI DMPT meets twice per month and 3 times per year face-to-face. In October, the group will invite participants from a number of countries to discuss detection methods. DMPT's goal is one detection method should be used for one product globally. DMPT also promotes greater global harmonization in regulatory systems and policies regarding detection methods, sampling, reference materials for product registrations and to enable trade. The DMPT also helps define and specify conditions to preserve intellectual property rights in reference materials and detection methods.

In Russia, detection methods are an emerging market and DMPT has reviewed and provided feedback on the national detection method draft. The DMPT has also shared currently available methods for detection of GM crops and some practices employed globally.

In the EU, the DMPT works with EuropaBIO on issues. They have worked to assert intellectual property rights by reviewing EURL disclaimer language and publishing a position paper on why detection methods should not submitted to ISO. The DMPT has also reviewed and provided comments on the EU Minimal Method Performance Requirement. DMPT has persisted in the position regarding warranty and to obtain clarification on SANCO's position on AOCS certified reference material terms and conditions and is having ongoing discussion through EuropaBIO with SANCO agreement that CLI Legal will draft new language.

In China, the DMPT supported a detection methods workshop and inspection seminar. The group also supported CropLife China to help present a workshop on the safety assessment and detection methods for GM crops. This workshop afforded interactions with key detection methods experts from the Ministry of Agriculture and AQSIQ/CIQ. The DMPT also reviewed and provided comments on China's new guidance document on detection method requirements and worked with CropLife China, Ministry of Agriculture and USDA to address the viable seed requirement as reference material in China. They also obtained CLI member company agreements on a proposal to participate in development of GMO screening methods by AQSIQ/CIQ.

In Taiwan, the DMPT provided an industry aligned position on detection methods for Taiwan FDA and Council of Agriculture.

DMPT has its own website (http://www.detection-methods.com/) which provides access to detection methods for seed companies. The group has updated and released a new version of the detection methods database and have continued their effort to link the DMPT website with the Biosafety Clearinghouse site. DMPT position documents are also published on the website. DMPT is constantly monitoring countries for emerging issues such as EU EFSA's sampling mandate, i.e., field sampling. The DMPT also has a country detection methods spreadsheet. The group is in discussion of how to share detection methods for pre-commercial products and is considering a proposal for a global detection methods symposium in 2015 to bring detection methods experts from key countries together.

CLI Food Feed Project Team (G. Shan, Dow AgroSciences): The CLI FFPT was formed in 2014 to act as a liaison between CLI and ILSI CSAFF (Center for Safety Assessment of Food/Feed). Other goals include developing and advocating regional and global positions on risk assessment and advocacy for food/feed safety of biotech crops and serve as a resource to the CLI Communications Committee, global network and other relevant CLI committees and project teams.

There are many challenges for food/feed safety assessment. For molecular analysis, there is inconsistent criteria for bioinformatics analysis in safety assessments; how to characterize products from new breeding technology and assurance of lack of off-target effects; how to deal with SNPs and the inconsistency of sequence results from re-sequencing of individual events in a stacked trait product; and how should new generations sequencing approach be used in molecular characterization. For protein analysis, the questions revolve around the selection of crop tissues for expression analysis and data transportability of already approved protein characterization for proteins that are well known such as PAT.

For whole food/feed risk assessment, is there a routine need for 90 day feeding studies on rats for both single events and stacked products of those events; the issue of duplicate feeding studies within country labs; routine need for animal performance study (such as broiler chicken) and herbicide residue trials for herbicide tolerance traits.

Currently, the FFPT is working on global data/study harmonization across technology providers by leveraging CLI technical teams and coordinating with the CLI Plant Biotech regional network.

CLI Stacked Traits Project Team (P. Hunst, Bayer CropScience): The Stacked Traits Project Team (STPT) reports into the Plant Biotech Regulatory Steering Committee (PBSC) of CLI. Representatives of the member companies BASF, Bayer CropScience, Dow AgroSciences, DuPont Pioneer, Monsanto and Syngenta make up the STPT. The STPT advocates a) no additional regulation of conventionally-bred stacked trait products, b) science-based regulations, if a country chooses to regulate, c) scientific knowledge of the parent events as a basis for potential interactions when stacked, d) the safety assessment of the highest order stack product applies to all smaller commercial sub-stacks, and e) harmonization of regulations and data requirements globally. The STPT has published position papers which are available on the CLI website as well as participating in workshops on stacked products in countries such as China, Korea and Southeast Asia countries. Stacked trait products were planted on over 99 million acres globally in 2014. In U.S. corn, stacked trait products accounted for 76% of the corn acres in 2014. Regulation of stacked products varies globally from notification letters to full safety assessments as is done for single parent events. There are challenges for breeding stacks, particularly around data requirements. In the EU, *de novo* sequencing of the stacked products is a requirement which is not always easy when the stacked product contains single parent events from more than one technology developer. Another challenge is the presence of sub-stacks in the grain of stacked products from segregating crops (such as corn). Industry advocates that scientifically there is no reason to assess sub-stacks when the highest order stack has been fully assessed. However, how can this be technically approved? The CLI DMPT has issued a statement that quantifying stacked products in bulk shipments cannot be done. The EU JRC Working Group has also issued a similar statement. Does AEIC agree with this? Is there any theoretical or experimental approach not yet explored? Cross-reactivity in large stacks (4 or more events) may be higher which presents detection challenges. Can AEIC members suggest any solutions that may be on the horizon for this? There is also a need for standardized assay validation/method transfer processes to help increase the ease of assay transfer. Would AEIC be interested in publishing an updated guidance for this?

ILSI CERA and CSAFF (M. Wach, ILSI): For those countries that are signatories (170 countries) to the Biosafety Protocol (BSP), there is a commitment to have risk assessment process in each country. The U.S. has spent about $135 million to help countries put a risk assessment process in place. Risk assessment should be science-based, using recognized risk assessment methods. Risk assessement entails comparing the GM crop to the non-GM crop and is case-by-case but is informed by all relevant data. There are several challenges for risk assessment:

- How to structure the process in a logical, efficient, transparent way
- How to identify relevant data
- How to know when you are done which is a struggle for regulators and risk assessors

Idenfiying relevant data involves using the pathway to harm framework. This focuses on a specific risk hypothesis and breaks a big question down to a series of causally related steps. In the process, relevant data is identified which is need to address the likelihood of each step occurring. This all helps in writing final decision documents.

The GM crop database is located on the CERA website (http://www.cera-gmc.org/GMCropDatabase) and provides a profile of commercial events. It is useful to new countries to help them understand the risk assessment of events and risk communication.

The Crop Composition database (www.cropcomposition.org) allows regulators (and others) to see the range for analytes across the species. Currently, there are 140,000 data points for six different crops in the database. There are no values for GM crops in the database, however.

CERA (Center for Environmental Risk Assessment) currently has protein monographs which provide comprehensive profiles of various proteins (CP4 EPSPS, Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry3Bb1, Cry34/35, PAT, Vip3A). Web-based learning tools have/are also being developed for risk assessment for low-level presence, problem formulation in environmental risk assessment and concepts of food safety assessment (in development). CERA has also organized a NTO testing workshop which gave participants lectures, labs and field testing experience. This was done in cooperation with DuPont Pioneer.

More information about ILSI is available at www.ilsi.org.

Patterned Flow Cell Technology and HiSeq 3000/4000 (P. Bathmaier, Illumina): The patterned flow cell has billions of ordered wells with nanowell substrate. There is optimal fixed cluster spacing and simplified imaging. Kinetic exclusion amplification delivers single (monoclonal) template per well which allows simultaneous template hybridization/amplification. Amplification occurs at a 20X rate. The flow cell is an eight channel cell with dual surface imaging. Each cell has template primers (oligos). The DNA comes into the cell and hybridization starts with standard SPS sequencing following. The samples are DNA, targeted DNA, or RNA. The system works well for all. The sequencing systems are the HiSeq 3000 or 4000. The 3000 instrument can generate up to 750 gb data per flow cell in 3.5 days using a single flow cell. For the 4000 instrument, 1.5 tb data are generated per flow cell in 3.5 days using a dual flow cell. Twelve human genomes can be run in 3.5 days. The quality is very good. For the 3000, 2x76 = 46 gb/lane which yields 75% reads coming at Q scores of >30. For the 4000, 2x151=93 gb/lane which yields 75% reads at Q scores of >30.

Targeted Mutagenesis in Rice and Maize using TALENS and CRISPR/Cas9 (B. Yang, Iowa State Univ.): Nuclease-based DNA double-stranded breaks for targeted genetic modifications allows precise modification of a site. There are several technologies for this such as a) Zinc finger nucleases (Dow), b) TALENS and c) CRISPR. The breaks can be repaired by nonhomologous end joining which is not a precise process. Homologous base repair occurs when donor DNA is provided which matches part of the endogenous site plus the desired change. All these processes depend on site recognition: Zn finger is based on protein and TALEN and CRISPR can use protein or RNA to recognize DNA site. TALENS and CRISPR are used for many species of plants, animals and fungi.

Using rice as an example for TALEN and CRISPR, first callus is initiated → nuclease gene transfer →selection →regeneration → plant. The TALEN gene is designed and delivered via Agrobacterium. For selection of transformed cells, hygromycin resistance is used. The desired DNA is integrated into the genome. The genome can then be sequenced and no T-DNA from Agrobacterium will be found. This process was used to look for resistance to bacterial blight of rice caused by Xanthomonas oryzae. The S gene causes the disease and the bacteria delivers the protein to the plant cell. The protein target in the plant cell are the SWEETs (sugar transporters), thus the bacteria hijack the cell sugar process. The plant defense are resistance genes which are mutations in SWEET11 (xa13) and SWEET13 (xa25). These mutations occurred in the promoter region. A protein is designed for the mutation sites which can be inserted using a double-strand break process. The T-DNA sequence can be removed from the T1 TALEN-modified rice plants through crossing and segregation. TALEN-induced changes in rice SWEET genes confer broad spectrum disease resistance.

In wheat, simultaneous editing of three homo-alleles in hexaploid wheat confers heritable resistance to powdery mildew. TALEN technology allows moving the mutation in one step.

In using CRISPR, the Cas9 endonuclease plus guiding RNA is the complex which identifies the specific DNA site allowing the endonuclease to create a double-stranded break. Twenty nucleotides are needed for the reagent (guiding RNA). Mutations can then be generated and tested. A combination of Cas9 and single RNA also induces a large chromosomal deletion. Cas9/RNA complex can work in several places at the same time. Each construct produces high efficiency mutations in T0 lines.

In summary, TALEN technology has been used to generated disease resistant rice lines. TALEN is an efficient genetic tool for basic research to help understand gene function. CRISPR/Cas9 is highly efficient process in targeted mutagenesis.

In 2014, Celectis inquired of USDA whether their TALEN product was regulated. USDA confirmed that the product was a non-regulated article since there was no T-DNA (i.e., Agrobacterium sequence) present.

New Breeding Technologies: Regulatory Implications (S. Thenell, Thenell & Associates): New breeding technologies (NBT) are making the news and workshops are/have been held to discuss the implications. NBTs include a) genome editing via site-directed nucleases (Zinc finger, TALEN, CRISPR, meganucleases) or oligonucleotide directed mutagenesis (ODM), b) cisgenesis, intragenesis, c) RNA-dependent DNA methylation (RdDM) and d) reverse breeding, grafting.

Precise genome editing is achieved by harnessing double-strand DNA break repair pathways. These include non-homologous end joining (targeted mutagenesis, targeted gene knockouts, deletions, inversions) and homologous recombination (using a donor molecule to deliver DNA of interest to a specific site). Site-directed nucleases are specific to sites in the genome where they implement a double-stranded break. Site-directed mutagenesis is classified as SDN-1 (targeted mutagenesis or DNA excision), SDN-2 (targeted editing of a genome sequence) or SDN-3 (targeted gene addition). Gene knockouts can be used for crop improvement such as the removal of toxins (ricin in castor bean) or anti-nutrients (typsin inhibitors in soy). Knockouts can change antigenic determinants such as immunogenic epitopes in peanut or reduce phosphate in animal waste by knocking out gene in phytate biosynthesis. Knockouts can also be used to improve nutrient composition such as in oils or product quality (reduce browning in potato). Gene targeting is used for more complex traits such specialty oils for human consumption, fuel or lubricants by changing the fatty acids or by making secondary metabolites which can be used in pharmaceuticals and specialty chemicals.

Global regulatory systems for GM products are variable in that only certain countries have functional systems and there is no harmonization of regulations. Countries also differ in pre-market approval data required, labeling and post-market surveillance of the products. The EU has held meetings and workshops beginning in 2007 to establish a working group to discuss the risk assessment for the technologies. In the U.S., USDA published their first opinion on an NBT product for Scott's Miracle-Gro glyphosate-tolerant Kentucky bluegrass. In 2012, USDA APHIS established a procedure for Regulated Letters of Inquiry to increase transparency and predicatability. In 2015, USDA APHIS rendered 6 decisions on organisms by NBT processes. Other countries such as Canada, Japan, Australia/New Zealand, Austria and Germany have issued reports or opinions on NBTs.

The risk assessment paradiagm is robust and broadly applicable to NBTs. Modern analytical methodologies can detect nearly all genetic alterations, however, not all genetic alterations can be identified (SDN-1,2,3, ODM, RdDM) or distinguished from naturally-occurring alterations. Comparative risk assessment still require analytical methods for safety such as composition, toxicity, allergenicity assessments. Traceability and intellectual property are challenging for NBT use.

In summary, genome modification tools are powerful and being used more frequently. The regulation of NBTs is complex and involves both process and product criteria. Authorities should be pragmatic and not regulate what cannot be enforced.

New Member Presentation: JR Simplot (A. Fisher): JR Simplot is privately held company located in Idaho. The biotech program was started in 2001 with the mission to create Innate potatoes with only potato elements, i.e., "all potato in potato". The Innate technology uses no foreign genes, no antibiotic resistance markers and no backbone sequences, however, the technology is not fully cisgenic. Innate potatoes received USDA (2014) and FDA (2015) approvals.

Attendees at 2015 Spring Meeting

Agdia
AOCS
BASF
Bayer CropSciences
Critical Path Services
Covance
Dow AgroSciences
DuPont Pioneer
EnviroLogix
Eurofins BDI
Eurofins
Eurofins Nutrition Center
ILSI CERA
Iowa State University
Lynx Diagnostics
North Carolina State University
Oilseeds Consulting
OMIC USA
Romer Labs
SGS
Thenell & Associates
USDA GIPSA

AEIC Multiplexing Method Validation Working Group
Meeting Minutes

Prepared by:	Guomin Shan
Date:	14 April 2015
Reviewed by:	
Status:	DRAFT
To:	AEIC Multiplexing MV WG

Title: **AEIC Multiplexing MV WG**
Date: **14 April 2015, 5:00pm-6:00pm PST**
Dial in: **Webex**

Participants

David Levin ..... Covance

Sharon Settlage ..... Critical Path

Fred Claussen ..... EPL

Matt Cheever ..... Bayer CropScience

Ning Zhou ..... Dow

Guomin Shan ..... Dow

Helen Mu ..... BASF

Carl Maxwell ..... DuPont Pioneer

Lucy Liu ..... Monsanto (called in)

Absentees:

Michele Yarnall ..... Syngenta

Grant Yeaman ..... Monsanto

Yelena Dudin ..... Monsanto

Bibo Xu ..... Primera-Corp

Background

The purpose of this project is to develop consensus acceptance criteria for the validation of multiplexing quantitative analytical methods for GM proteins through surveying laboratories and institutions across

the Ag biotech industry. Technologies will be covered in this project are ELISA, MSD, Luminex and LC/MS. Example criteria are sensitivity and dynamic range, accuracy, precision, specificity, and equivalency. The expected result is to generate one or two publications in a peer reviewed journal, which can serve as an industrial guidance or consideration for method validation of multiplexing quantitative technologies for GM proteins.

Progress update: Survey of validation criteria for ELISA and LC/MS (initiated by Dr. Larry Mallis of Critical Path) is completed. The original survey is attached. The manuscript has been drafted by Sharon and is circulated among members (Title: Acceptance Criteria for GLP Validation of Quantitative Analytical Methods for Proteins by ELISA and LC-MS/MS: Survey Results). First round feedbacks were collected.

Plan for the survey paper (Validation criteria for ELISA and LC/MS)

The team has agreed to have the ELISA and LC/MS survey paper as a stand alone publication.

1. **Sharon** will send manuscript to Helen and Matt for review comments. **Helen and Matt** to provide feedbacks in one month.
2. Once feedbacks from Helen and Matt are received, the final version manuscript will be sent for approval by each member. **Note**: as discussed during AEIC business meeting, the final manuscript will be sent to the AEIC Board for courtesy review, and AEIC will be acknowledged in the paper.
3. Proposed journal to submit: Food and Agricultural Immunology (FAI).

Plan for multiplexing paper

The multiplexing technologies team members experienced include MSD, Luminex, and LC-MS/MS. Some members have not had a chance to explore multiplexing technologies yet. The key method parameters for single-plex assays, which have been surveyed and discussed in the first paper, apply to multiplexing assays. However, each assay parameter has different challenges in each multiplexing technology/format. The focus of the second paper will be on the difference and considerations of challenges of each parameter when it applies to multiplexing platform. Team agree to start with a survey, and then have one member with practical experience on multiplexing method validation to draft a manuscript. Other team members will contribute by reviewing the manuscript and providing comments.

1. Outline of survey: use the same parameters as single-plex method and populate a list for survey. The content will include the difference and main consideration for multiplex method for each validation parameter. **Sharon and Guomin** will draft a survey list outline. Timeline: 1 month
2. Collect survey result from each participant and summarize the results.- **Sharon** will lead to summarize the results. Timeline: 3 months.
3. Draft a paper based on survey results – Since Monsanto scientists have experience on both MSD and Luminex technology, **Monsanto team** will draft the manuscript; - Timeline: 3-6 months (**Lucy**).
4. Collect review input and feedbacks from team for final draft of paper.- Timeline: 3 months. WG will meet at 2016 AEIC Spring meeting to review the progress.

AEIC Meeting - Composition Working Group

Tuesday, April 14, 2015
14:00

April 14; Embassy Suites - La Jolla

Welcome, Introductions and Antitrust Statement (Gina Clapper)

- Introductions
- Reading and viewing of Industry Statement for Anti-Trust Compliance

Update on ELLA Lectin Method (Gina Clapper, AOCS)

Verbal update, slides to be provided for incorporation into the minutes

Background

- Elisa Leyva-Guerrero reviewed some background.
- Introduce new methodology compared to hemagglutination method.
- Similar to ELISA method with commonly-used tools.
- Collaborative project through AOCS
- Samples were delivered everywhere intended except Brazil (have US, India, China)

Publication update (JAOCS)

- Was able to get a 2nd reviewer and reviews have been returned.
- Comments are to be reviewed; may reach out to authors for additional input.
- Expect publication soon.

Statistical analysis

- CVs not as close as desired (~20-25%)
- Other ELISA-type methods have similar (25% CVs), CVs look good compared to hemagglutination lectin method.

Path to Acceptance as an AOCS Method

- AOCS Seed and Meal committee for vote for incorporation as a method
    - Methods committees prepared for receipt of this new method.
    - Will have some language putting the CV in context (not a familiar 5-10% CV).
    - No anticipated vote difficulty over 6 week voting period.
- Method will go through AOCS Uniform Methods Committee then; anticipated Summer inclusion as a method.
- Will be out for vote during the AOCS meeting.

Questions

- Anticipate another publication after the method is released. Not sure if collaborating labs will be authors of the publication (experience has been that the participating labs are listed in acknowledgements).
    - Main sites from each company could be included as an author?
    - Group that developed the method listed as authors (minimum)?
    - AEIC Working Group members to let Gina (gina.clapper@aocs.org) know their thoughts by 2015-MAY-15.

Update on Cyclopropenoic Fatty Acid in Cottonseed collaborative (Gina Clapper, Barb Mitchell)

Introduction (Gina)

- Call for participants in AOCS newsletter in mid-March; 5 responses.
  - Regulator (CFSAN) wants to participate.

Manuscript Status (Barb)

- On 2nd revision; hope to resubmit by the end of the month.

List of Participants (Barb)

- 10 laboratories
  - Eurofins - one lab (maybe 2 - Keith Persons spoke with India - interest in participation)
  - Monsanto - one lab (maybe 2)
  - Covance - 2 if not 3 labs
  - EPL – one lab
  - 5 labs from AOCS call for participation.
- Target for minimum 12 labs in anticipation of removing outliers or inability to distribute samples. Close to target.

Protocol Review

- Formal not been written yet, but it is in AOCS form.
- Need minimum 5 samples with blind duplicates. In process of determining if they cover the best range and selecting ones that provide the most breadth.
- Sources:
  - Morgan sent 10 cottonseed samples to evaluate for use in collaborative samples. Some variation but not a lot.
  - Refined and unrefined oil.
  - Tilia oil (form of linden tree oil as a standard): Linden blossom oil.
  - Looking for another strain of cottonseed (accounting for 5% of the cotton crop) - additional source of variation.
  - Bayer - has some sources of germplasm that may support breadth (Gillikin).
    - Gillikin working to acquire and will send to Barb once she has some (as a pre-assessment to determine if it could be included).

Standards

- Covance has Dihydrosterculic acid ME standard
- Manufacturing standards update for Sterculic and Malvalic
  - Matreya
    - Sterculic standard at 80-90% purity - $10k for 100 mg (anticipated a couple of grams). Would come with certification; with what backing?
    - Malvalic - work is starting to synthesize.
      - $5k for 100 mg
      - Request more with some sponsorship (below)?
    - Recommend that we characterize the purity independently. What do you use to assess? (Area % with mass spec for identity). Expect to use a couple of milligrams for the assessment.
  - BOC Sciences
    - BOC Sciences - $2k for 100 mg
    - BOC Sciences - $10k for gram of Malvalic

    - Covance to receive and distribute?
- Support for purchasing standards
    - Sponsorship
        - Would Cotton, Inc. sponsor a purchase?
        - Participants pay a participant fee?
- Standard would only be for the collaborative and generating solid data on response of these standards
- We do not anticipate standards would be used routinely for the method (increases costs). Write information into the method (response factor in method to support method application).
- Would be good to get both standards considering the work invested in measurement, but would accept one and would be possible to move forward without these – use closely related standards to benchmark relative retention times and response factors

Next Steps

- Barb to go back and talk with Matreya about pricing and timing
- Draft of a proposal to cost-share standards.
- Gina to solicit Cotton, Inc. for sponsorship

Gossypol co-collaborative

- Mike had received cottonseed samples sent to make up collaborative. Was running in his lab; taken a little longer with type of seed sent.
- Gina to talk with Mike Dowd at AOCS and communicate back by 2015-MAY-15.
- Needs discussion of how much samples overlap; also has access to other sources to support.
- With participation list, if most are interested in both, then include it to simply sample handling.

Fat Soluble Vitamins Project Status (Fred Claussen, EPL Labs)

Slides – included in mailing

Objective review

- Combine K1, Vit E, and beta-carotene into one method; improve accuracy/precision/turn-around/inter-lab harmonization.

Method scope

- Soy includes all 3 (a, b, gamma, delta tocopherols, vit K1, beta-carotene) (B-carotene should not be present in mature soybeans - only seen above LOQ for 5% of samples).
  Broad range of tocopherols in ILSI database.
- Canola - no beta-carotene
- Maize - no vit K1 but wide range of beta-carotene (attributed to methods)
- Cotton - only alpha-tocopherol present; not a primary crop to address with methods.

Proposed Determinative step

- Current Methods in ILSI database reviewed.
- Proposed: HLPC-MS/MS with electrospray ionization, HPLC parameters, linear range.
  HPLC-MS/MS: advantages over other methods ("universal", sensitive, selective, and available). Hardware is dependable and robust with good linear range (100 expected; 500-1000x sometimes). Disadvantage is that they are prone to matrix interference and multiple ionization mechanisms for non-polar compounds (w/ESI)
- Detection parameters presented
  Tested with older equipment - presents the best case for method transfer to other labs.
  Example of beta- and gamma-tocopherol - need good chromatography because of near identical MW and mass/charge ratio.

- Chromatography
    - PFP = Pentaflourophenyl Propyl
    - Preferences and compromises
        - Is detection of isomers important? Not required by OECD; cis produced by stress during processing or exposure to heat, light, etc. (trans is biologically active)
        - Leaning toward PFP and down-prioritizing need for isomeric resolution.
        - Specific conditions (column, mobile phases, and gradient conditions) presented.
- Linear Range
    - Normal to determine purity based on known extinction coefficient of vitamins (spectrophotometry).
    - Currently just using COA and serial dilutions to assess method performance during development process.

Proposed Extraction

- Aqueous hydrolysis vs. solvent extraction.
- Combined extraction procedure considered (endogenous seed vitamins vs. fortification). Hydrolysis is needed to release endogenous FSVs.
    - Alkaline Hydrolysis will destroy Vit K1.
    - Lipase digestion - not enough to extract Vit K1.
    - Binary solvent extraction (DMSO:Hexane) from USP - extract encapsulated vitamins.
- Proposal:
    - Use DMSO:Hexane extraction procedure. Details of the extraction presented. Add BHT to hexane to prevent oxidation of tocopherols and beta-carotene.
    - Clean up with silica SPE to remove the extracted fat.
    - Sequential extractions tested:
        - With heat/sonication
        - Subsequent with room temp extraction. After 3 (total) extraction; very little additional Vit K1 recovered with 4th or 5th extraction
- Challenges
    - Overlap with beta and gamma tocopherol (when one is disproportionately high) - soybean example
- Review of Precision (soy lot control charted over a year) and to historical data

Looking Ahead for single lab validation

- Challenges with matrix suppression with ESI? Test with APCI? (Atmospheric Pressure Chemical Ionization)
- Consider stable-isotope internal standards; some challenges with tocopherols (synthesis could be expensive)
- Limited value of spike/recovery due with endogenous vitamins that require an extraction
- More optimistic that a method can be finalized.

Questions

- Expecting coelution of cis- and trans- beta-carotene with both columns. Push with dichloromethane may be pushing everything through the column together. Also may be a challenge with other pigments/carotenoids with corn.
- Subteam - Fred to head up a subteam.
    - Close to a point that other CROs might support method development? Willing to receive help.
    - Are other CROs willing to support method development? There is interest by Eurofins and Covance.

Allergen Update/Discussion (Morgan Petty, Monsanto)

Slide presentation

Allergy Subgroup update (Morgan)

- Assessment of endogenous allergens for GM foods (beyond introduced proteins).
  - Introduced protein - bioinformatics similarity to known allergens
  - Endogenous allergens - almost like composition work
- Existing methods of endogenous allergens - human sera from allergic individuals (like lectin or hemaagglutination methods)
  - Ladics et al. Reg, Tox, & Pharm 70(1):75-9.
- Method summary: Gel separation, Mass Spec, or ELISA
- Two calls from Allergen Subteam
  - Broad use of method: most are ELISA or Mass Spec (most of industry)
  - First call
    - Harmonization - two groups with established methods. Harmonization was tabled. Potential to use round-robin - at least understand differences in results.
  - Second Call
    - More depth with methods.
    - Legal concerns with sharing natural variability data with non-GM samples. Still being evaluated and legal teams in consultation, possibly with more generic assay performance data.
- Continue to use sub-team for differences in methods.
- Allergens are only required in Europe and the review must only be to the reference lines (not a broader review of natural variation). Cannot do a retrospective review for support from ILSI database, for example.

Composition Working Group Mission/Charter (Carl Maxwell, DuPont Pioneer)

Carl (slide deck)

Why Collaboration (historical perspective)

- AOAC - cost prohibitive and wanted to bring in regulators earlier
- AEIC with AOCS backing supportive and made Comp Working Group Possible
  May need other group support for some methods (e.g. water soluble vitamins)

Mission Statement

Words

- Composition - what does this mean to the group?
- Analytical -
- Regulatory -
- Ag-Biotech -
- Collaboration - diverse set of interests

Increase quality while meeting regulatory requirements.
Public good - reduction in cost with improved quality (enables universities and institutions)
Reviews of historical data for harmonization (ILSI DB).
Methodologic safety

Mission Statement composed and presented during the AEIC meeting. (see attachment)