AEIC Fall Meeting 2012
Meeting Minutes
October 3-4, 2012

P.L. Hunst, AEIC Secretary

The AEIC Fall Meeting 2012 was held in Portland, Maine on October 3-4 and was hosted by EnviroLogix, Inc. There were 46 registered attendees. J. Markin, President and CEO of EnviroLogix, welcomed the group to Portland and gave a short presentation. EnviroLogix, Inc. has been in business for 16 years and was acquired by Ensign-Bickford Industries in 2010. Ensign-Bickford Industries has a broad portfolio of businesses which includes aerospace defense, pet food, chemicals, etc. EnviroLogix is headquartered in Portland and was recently awarded the Portland Business of the Year for 2012. The business has platforms in immunoassays, DNA kits, mycotoxin lateral flow devices, QuickScan strip reader, rapid isothermal DNA assays, etc.

AEIC BUSINESS MEETING

Spring Meeting 2012 Minutes: A motion was made, seconded and voted positive to approve the minutes that are on the AEIC website.

Treasurer Report:

2012 Budget

	Projected	**Actual**
Beginning Balance	$35956.00	$35956.00
Dues	7200.00	10800.00
Interest	25.00	24.00
TOTAL	43181.00	46780.00
EXPENDITURES		
Scientific Paper	7000.00	3000.00
Wire Transfer		
DE Franchise Tax Report	25.00	25.00
Board Meeting	125.00	136.00
ISO	2900.00	
2012 Spring Meeting	1500.00	4090.00
AEIC Website	350.00	271.00
Bank service charge		10.00
2012 Fall Meeting	2500.00	900.00
Graphic design		
Reprints	800.00	
Subscriptions	100.00	
Miscellaneous	100.00	
TOTAL	15400.00	8431.00
Balance (after expenditures)	27781.00	38348.00

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

AEIC Member Update (D. Layton):

	Number	Potential Dues	Unpaid Dues
Large Companies	13	$6500.00	$500.00 (1)
Small Companies	13	3250.00	500.00 (2)
Associates	2	100.00	
Individuals	2	200.00	
TOTAL	**30**	**10050.00**	**1000.00**

Meeting Registration Discussion (C. Alarcon): AEIC meeting costs are going up which may exclude smaller companies from hosting a meeting. It was proposed that AEIC might consider charging a meeting registration fee to help defray the meeting costs. Registration fees are common for most other types of meetings. The question was asked as to what AEIC is planning to do with the funds already in the treasury? AEIC should be doing more to justify the $30,000+ in the treasury. Should AEIC wait until the treasury hits a minimum "cushion" and then again discuss registration fees and/or increase membership dues? If meeting registration is charged, would this discourage participation by government agencies and other groups? It was commented that manpower for planning and executing a meeting was more of an issue for any member company, regardless of size. The fundamental question is whether AEIC needs more funds. It was decided to table further discussion on a meeting registration fee until when and/or if more funds are needed by AEIC.

Brochure Update (D. Layton): Dean is currently waiting to reprint brochures until feedback is given by the members. The brochure is posted on the AEIC website.

Spring Meeting 2013 (C. Alarcon): Three member companies volunteered to host the meeting: Covance in Madison, WI; AOCS in Champaign, IL; and Agdia in South Bend, IN. It was suggested that the membership should vote via e-mail as to which location would be preferred. The AEIC Secretary will send out a voting ballot.

Suggestion for possible topics:

- Genoplasty
- High throughput analyses
- New methods for lectin analysis
- Stealth GMOs
- Proposition 37 in California (if it passes)
- AP testing for trait development and discovery
- Reference material
- Botanical impurities (example: GM corn in soybean products)

AEIC Vice President Election: Nominations were opened for the office of Vice President on the AEIC Board. The Vice President serves one year and then transitions to the President for a year. After the term as President, the person then transitions to Past President on the AEIC Board for a year. Thus, the office is a three year commitment.

Denise Thiede (BioDiagnostics) and Darren Cook (Douglas Scientific) were nominated during the meeting and both accepted the nomination. The AEIC Secretary will send out a notice announcing the opening of nominations in October. Voting will be via e-mail to begin in early November.

AEIC Publications:

Laura Privalle announced that the paper on biotech event development and testing was accepted for publication in the J. Food and Agricultural Chemistry.

Clara Alarcon indicated that the paper on protein and DNA methods is still in the draft phase and is still missing one section from a contributor.

Clara also indicated that Beni Kaufman is still working on the paper on quantification by sub-sampling and the use of SeedCalc.

AEIC Goals and Activities (C. Alarcon): It was suggested that AEIC may want to consider doing another workshop on detection, possibly with USDA GIPSA. Topics might include practical applications, market realities, etc. Tandace Bell (USDA) indicated that this might timely, however, USDA has a lack of resources currently to fully coordinate.

Another suggestion was putting together a video for YouTube to show the importance of GM products for agriculture/food production and the testing that is utilized.

Gina Clapper indicated that AOCS is hosting a workshop on lab methods in Ankeny, IA. The workshop is currently all chemistry oriented but it may be worthwhile to consider a section on protein/DNA detection for GM products. Gina will send the workshop brochure to the Board for consideration (see attached).

UPDATES

ISO/TC 34/SC 16 (G. Clapper): TC 34 deals with food products and SC 16 works on molecular biomarker analysis. Final draft revisions have been made for proteins. The 4th Plenary Meeting will will be held April 8-10, 2013 in London. One of the work items has been requested to be dropped by France.

Business was adjourned.

INVITED TALKS

Global Population is Increasing, Arable Land is Decreasing, Technology Must Provide a Solution (L. Privalle, BASF)

The global population is projected to 9 billion by the year 2050. The US provides 18% of the world food supply from 10% of global arable land. One US farmer produces food for 155 people. To meet the food demand of the growing population, crop yields must increase 2-3 times to meet the demand. Corn yields exhibited a big increase in the 1990's due to the introduction of biotech crops. In Kenya, by contrast, yield has stagnated due to minimal agricultural inputs. Biotech crops are just one solution. Genetics will need to continue to be improved using marker-assisted breeding. Also, the continued development of good, friendly, environmental chemical solutions must proceed als as well as increased acceptance of biotechnology and solving food distribution problems.

Biotechnology contributes to sustainability as well as providing other benefits. Rapid adoption of biotech crops has occurred. More than 10% of crop land is used for biotech crops (15.4 million farmers growing biotech crops in 29 countries). Ninety-percent (90%) of biotech crops are cultivated by small acreage, resource-poor farmers. The countries planting the highest percent of biotech crops are the US, Brazil, Argentina, India, Canada, China, Paraguay, Pakistan, South Africa and Uruguay. In the US, herbicide-tolerant sugarbeet was the most rapidly adopted biotech crop (>95% adoption in 3 years). Eighty-eight percent (88%) of corn and 90% each of cotton and soybean acreage in the US are biotech lines/varieties. Stacked trait products are cultivated on 49% of US acreage. Globally, 50% of corn, 70-80% of soybean, 20% of canola, 30% of cotton and 5% of sugarbeet grown are genetically modified (GM). So the question is: What is "conventional"?

Corn yields have increased by 15% since the 1990's with the introduction of the Bt insecticidal genes into corn lines to control the European corn borer pest. Herbicide tolerant varieties dominant the soybean varieties that are grown. Blight resistant potatoes carry resistance genes from wild potatoes. These potatoes were originally projected to be launched in the EU in 2015, however, this is now unrealistic as the developer has chosen to leave the EU for plant research/development. Another success story is the introduction of GM papaya in Hawaii which is resistant to papaya ringspot virus. This has saved the industry in Hawaii.

GM crop benefits include increased yield, protection against pests and protection of the environment due to less use of pesticides. GM crops are evaluated globally for human and environmental safety before they are authorized for cultivation and use in food/feed. There are many political issues surrounding GM crops such as food labeling laws. Some countries have instituted thresholds allowing GM presence in food/feed:

- EU - 0.9%
- Japan - 5%
- Korea - 3%
- Australia/New Zealand - 1%

For most countries, there is 0% tolerance for unapproved GM traits (events).

History of US Food and Drug Administratin (FDA) (D. Layton, EnviroLogix): A century ago, there were no federal laws or regulations to protect the US public from dangerous substances in medicines and food. In 1862, Abraham Lincoln appointed a Chief Chemist and in 1880 a proposed national food/drug law was defeated in Congress. In 1883, Chief Chemist Wiley investigated the use of preservatives and expanded this investigation to broader food adulteration. In 1906, the Pure Food and Drug Act was passed which was primarily prompted by poisonous substances being used in food. In 1907, regulations were instituted for food colors and in 1927, the Food, Drug and Insecticide Agency was established. The Black Book, guidance for industry, was published in 1949 and in 1958, FDA published the first list of GRAS (Generally Recognized as Safe) substances. Sanitation programs were instituted in 1969 and low-acid food processing regulations were established in 1973. 1980 saw the passage of the Infant Formula Act and in 1990, nutrition labeling on food became mandatory. HACCP (Hazard Analysis Critical Control Point) regulations were instituted in 1995 to ensure safe and sanitary processing of fish and fishery products. The juice HACCP was instituted in 1998 which requires warnings on unpasteurized juices. In 2002, the Bioterrorism Preparedness and Response Act was passed and the Food Allergen Labeling/Consumer Protection Act goes into effect in 2006. The Egg Safety Final Rule was passed in 2009 and in 2010, the Food Modernization Act (FMA) was passed. The intent of the FMA was to be more proactive rather than reactionary to prevent contamination during food processing.

Monitoring and Assessing Food Safety Parameters: Contaminants vs Adulterants (M. Taylor – Texas A&M): Food safety challenges include enteric viruses, enteric pathogens on meat/poultry (such as Salmonella and E. coli) and pathogens on fresh produce such as viruses and bacterial pathogens. Enteric viruses include the Noroviridae which occur on fresh produce, shellfish and through fecal/oral transmission. These viruses have an incubation time of 12-48 hours. The hepatitis A virus is spread between people, via food and drinking water. Symptoms include diarrhea, jaundice, fever and abdominal pain and an incubation period of 15-50 days. Enteric viruses are the number one cause of foodborne disease (5.46 million cases/year). Commercial detection systems are available from Shimadzu, Invitrogen and Eiken (examples).

Enteric viruses are classified into 5 geno-groups. The pathogenic groups are 1, 2 and 4. Most detection is via RNA tests. In food, RT-PCR from food homogenate is used. This has limitations on sensitivity and processing of food. The NASBA (nucleic acid sequence assay) has a higher sensitivity than RT-PCR. Recently, there has been an ongoing outbreak of Noroviridae in Germany, i.e., 8400 children are sick in Berlin. These cases are potentially connected via a single food supplier, however, the food item has not yet been pinpointed.

Bacterial pathogens, such as Salmonella (non-typhoidal) and E.coli (shiga toxin), are a significant cause of foodborne disease. Multiple foostuffs have been implicated or observed as transmission vehicles. *Salmonella enterica* has a 12-72 hour incubation period post-consumption. It has ineffective growth at reduced temperatures or low pH (4.0 or below). However, it may survive at a low pH in some foods and retain its virulence. This is also true in reduced water content foods (peanut butter). There are 1 million new cases/year with about 380 deaths reported. Domesticated animals represent potential reservoirs. *S. enterica* is spread by human to human transmission. It can be controlled by proper cooking and food processing, low temperature storage,hygiene of food preparers and processing personnel. Recent outbreaks include peanut butter (ongoing), alfalfa sprouts (2009-2011), ground beef/poultry (2011-2012), salami (2010), tomatoes/peppers (2009) and almonds (2005, 2007). FDA uses biochemical and serologic testing which are long processes. PCR methods are used by companies but have not been fully embraced by FDA. Food Safety and Inspection Service (FSIS) uses a combination of biochemical and molecular screening. The key concerns for testing are a) the accuracy in detection, increased sensitivity/specificity; b) multiple serovars present in some foods; c) volument of sample processed; d) volume of sample processed; and e) need for concentrating pathogen from food sample.

E. coli O157:H7 has been found in ground beef and non-intact beef cuts. Detection of the pathogen results in disposal or diversion to full cooking processor. O157:H7, when detected, is considered an adulterant. The big 6 serotypes (STEC) drove the 2010 act. Ground beef, lamb, veal, sausage, raw milk-derived products identified as transmitters. Not all non-O157 STEC are known as human pathogens. Detection methodologies were not fully developed prior to the passage of the regulations. *E. coli* O157 is controlled at processing by raising the core temperature of meat, pasteurization of milk, irradiation of meat, organic acid use on carcasses. Also, sanitation in slaughter and processing is important.

Other pathogens of concern are *Listeria, Clostridium difficile,* methicillin-resistant *Staphylococcus aureus.* Testing needs include tests with a) sensitivity and specificity; b) user-friendly testing; c) gaining reliable data from the testing to make process decisions.

Food Safety Modernization Act (FSMA): Implications and Implementation Challenges (D. Levin, Covance): The US enjoys an amazing food supply with an array of every type of food availability year around. Fifteen percent (15%) of food consumed in the US is imported (60 million metric tons). In recent years, FDA has stopped about 100 shipments of food from China due to the presence of cancer agents, melamine, banned antibiotics and illegal pesticides. Salmonella is always a problem. The Centers for Disease Control (CDC) estimates that 48 million people contract a foodborne illness and approximately 3000 die. Many cases are unreported.

The FSMA is the most important food law in over 70 years in the US. It has taken 3 years to enact it and now FDA must write enforceable regulations. Testing is expected to increase to 10%. Enacting FSMA requires an additional $1.4 billion to administer. FDA can enforce interim rules.

The FSMA allows inspection of records which is a powerful tool for FDA. It also requires registration of food facilities, fees, eliminate port shopping by FDA reporting to Homeland Security when they reject a ship, inspections, mandatory recall authority and whistleblower protection. Since FDA cannot handle all testing, labs need to be accredited to assist in testing imported foods, supporting import alerts, identify food safety issues. Lab accreditation will also allow testing in country of origin or at borders. Accreditation is to be effective no later than 2 years following enactment. Model standards of FSMA include appropriate procedures, quality systems, complaint response, qualified trained personnel. Records are to be sent directly to FDA. FDA is working on a database structure.

FDA is to determine the most significant foodborne contaminants every 2 years and then issue guidance documents. For fresh produce, the authority is given to set commodity-specific standards and prioritize implementation. FDA has assigned 450 full-time equivalents (people) to FSMA. These positions are split among 7 implementation teams. FDA must interpret the implementation requirements, publish proposed guidance doucments, publish notice of proposed rulemaking and interim rules. Town hall meetings across the country have been completed for input. FDA was to receive $1.4 billion over 4 years, however, Congress is considering cutting the funding. Fines and accreditation costs will only yield $100 million/year so not enough to meet the implementation cost.

FSMA is now in effect and it is expected that FDA will be soon be publishing the rulemaking notification. Public comment on this notice is critical. FSMA updates from FDA are available via e-mail.

R. Bohannon (Agdia) has an available app for traceability called SampleTrax which is a chain of custody GPS-bar code app. It is an Android app that records date, time, user, GPS location or manual entry of location. The app allows the user to select what tests they want to run from a menu and also allows the user to photograph the plant/site/sample and upload to a secure site. The app and more information is available at http://apps.agdia.com/sampletrax/.

Biotechnology Pipeline of Traits to Address Demands for Increased Food Supply (M. Newell-McGloughlin, U. of California – Davis): Today, 17% of land currently under cultivation is degraded by human activity. Agricultural land has decreased by 20,000 hectares/year. Without crop yield increases, land use will double by 2050. In Latin America, the greatest yield increase had lower land use and less deforestation.

Humans have been modifying plants since 8000 B.C. These modifications have resulted in the use of chemicals such as colchicine to induce changes, i.e., asparagus was colchicine-treated and then anther cultured to obtain modern asparagus. Japan has a large program using cobalt^{60} to generate desired mutations in plant genomes. In contrast, genetic modification via biotechnology methods is a much more controlled modification and is more efficient.

To increase crop yields, all tools that are available need to be used. New tools available include a) next generation sequencing; b) comparative genomics transcriptome analysis; c) high density maps; d) RNAi; e) transcription factors; f) transcription-activator-like effect nuclease (TALEN); g) SNaPshot high-information content fingerprinting (HICF); h) chemical genomics; i) novel maternal/paternal haploid product (centromere engineering); j) mini-chromosomes; k) epigenetic modifications; l) network engineering; and m) systems biology reductive/holistic approaches to identification, modification, introgression and study of expression/interaction.

There are opportunities and challenges for GM crops. Most GM traits are input traits which benefit the growers. These include traits for biotic stress (pests, diseases, weeds) and abiotic stress (drought, heat, salinity, submergence, marginal soils), yield (nutrient efficiency, fossil genes). Quality traits improve post-harvest characteristics, shelf-life, processing, taste, improved nutrition, functionality. Plants have also been modified to be factories for pharmaceutical and industrial products. An example is rice producing lactoferrin lysozyme from Ventria.

GM crops have provided substantial economic gains ($80 billion between 1996-2010). Forty percent (40%) of the gain was due to reduced production costs and 60% due to substantial yield gains. In 2010, 76% of the total economic gain was due to yield increases. Use of chemical pesticides has decreased as well as the production of CO_2. In China, Bt rice has the potential to increase yields by 8%. Studies have shown that mycotoxins have been reduced in insecticidal resistant corn expressing the Bt proteins and that non-target insect actually fare better in Bt protein expressing crops than those treated with insecticides. Disease and insect resistance genes are being identified and engineered into plants. The Xa21 rice R gene confers resistance

to Xoo. The defense response is triggered by the Xo molecule, AvrXa21. The transgenic plant is more resistant due to copy number.

Abiotic stress is the limiting factor to crops reaching their genetic potential. Drought tolerant maize and canola are being developed. A new project in Mexico has identified some pathogens of maize that have now become commensal and thus, are fixing nitrogen for the maize. Other projects are exploring not just modifying pathways but whole networks within plants to improve functional benefits of plants. Omega-3 fatty acids such as delta-3 and delta-6 desaturase are being introduced into food. Omega-3 fatty acids are needed for brain development.

Analytical Approaches to Effective Food Safety Strategies (A. Mathew, Purdue U.): *E. coli* O157:H7 is a foodborne bacterial pathogen associate with cattle, deer and vegetables. In ground beef, the organism is internalized so if the ground beef is not cooked thoroughly, the bacteria can survive. *Salmonella* is the most common food pathogen that causes serious illness. More associated with beef since beef not always thoroughly cooked whereas pork tends to be better cooked thoroughly. *Campylobacter* thrives in a microaerophillic environment.

Foodborne illness occurs because there are more people in the world (315 million "eaters" in US). The population is more urbanized and thus, they have less contact with livestock bacteria and similar organisms. Thus, people are less resistant to bacteria. Pathogens in manure of a few animals may transfer to many animals during the farm to processor transfer. Manure may also contaminate equipment at the slaughter facility. The critical control points in this transfer are:

Farm → slaughter facility → processor → food handler → consumer

Zero occurrence of foodborne pathogens is possible but not likely. Foodborne pathogens are often natural residents of GI tract of animals. Vaccines or antibiotics are generally ineffective to control in animals. Pathogens have many animal hosts and are endemic in the environment.

HACCP became mandatory for slaughter facilties in 1995. This has found to be effective for these facilities. However, HACCP does not work from farm to table. There are many challenges to control from farm to table. The farm is an extremely complex, dynamic, intermixed inorganic/organic matrices. Surfaces are not all stainless steel/epoxy-based, non-porous composition. There is a continual influx of microorganisms. A farm cannot be shutdown and disinfected because chemical/physical disinfecting agents are too toxic to animals. Also farms have a minimal labor force. Downstream events may overshadow or confound farm pathogen mitigation. For example, animal mixing results in the pathogen detected on the farm is not the organism detected at the slaughter house. *Salmonella* moves into the animal lymph system and the animal can shed within 2 hours. Poultry, on the other hand, do not have animal mixing problems since the individuals do not mix but stay in crates until slaughtered.

The bioanalytical needs include the ability to detect and identify pathogens from complex organic matrices. Another need is to identify the pathogen to serovar/isotype level. This is needed to track the pathogen back to the origin through the complex microbial/animal/environmental dynamics. Structural, biochemical analyses are needed to enhance antigens that promote the optimal immune response. Novel antigens are needed for effective vaccines against invasive pathogens and gut-borne pathogens. Bacteriophages are specific to pathogen isotypes, thus, there is a need for phage-directed pathogen identification systems. There is also a need to identify competitive exclusion candidates, i.e., non-competitive microbial species used for competitive exclusion strategies.

Pre-harvest Testing of *Salmonella* and STECs in beef and poultry (V. Dutta, EnviroLogix): Between 1900-1930, tuberculosis (TB) was the biggest threat in the US. Ten percent (10%) of all TB deaths in children were due to consumption of raw milk. In 1917, pre-harvest and post-harvest of livestock began. The pasteurized milk ordinance was passed in 1927. Pre-harvest and post-harvest testing in 1942 was limited by the quality of diagnostic tools and by the

cooperatorion of livestock owners. The prevalence of the TB bacteria was reduced in cattle from 5% to 0.5%. Today, all efforts are concentrated in post-harvest testing which is guided by HACCP. HACCP has been mandatory in meat processing in 1998.

Foodborne pathogens are mostly commensal to the guts in livestock. Stress in animals can elevate food safety risk by causing transient colonization of pathogens. Most pre-slaughter meat contamination is traced back to the farm. High loads of pathogenic bacteria can stress meat production facilities. Pre-harvest testing provides cost effectiveness for food producers. There is inherent complexity in the structure of the pre-harvest arena since most farms are family-owned. There is a lack of diagnostic/intervention tools that give quick, accurate, meaningful results. The criteria for a good diagnostic tool include being able to conduct a hazard analysis, identify critical control points, establish critical control point monitoring requirements, establish corrective actions, establish recordkeeping procedures, establish proceduresfor ensuring HACCP system. Tools that are available are:

(higher specificity) DNA assays → antobdy assays → culture isolation and identification (least specific)

For PCR analyses, the major problem is sample preparation. The amount of DNA isolated is subjective. The *Salmonella* DNAble v2.0 assay is an isothermal, 10 minute assay in which 16 samples can be analyzed. The assay requires minimal sample preparation and can utilize crude samples. The result is available in 30 minutes. The sample can be enriched for 8-16 hours and re-assayed for confirmation. The sensitivity is 10^4 cfu/ml. The assay has been tested for cross-reactivity and was found to be 100% exclusivity and the test was found to be 100% accurate.

In the future, multiplex assays for STECs are in development. Also targeting other human pathogens in agriculture and human health.

Trends in Analytical Methods for Assessing Food Testing (D. DeMarco, Qualicon): The pathogen testing market rapidly grew in the early 2000's. In 2011, the market value is $3 billion. By 2017, it is projected that the market will grow to $4 billion. There is still a gap between current microbial diagnostic products and what is desired by customers. The trends driving the market include increasing regulations, demand for speed/simplicity, increasing public cases of recalls, consumer/media pressure, evolution of diet and high acceptance of molecular technology.

In the US, food pathogen requirements for meat, poultry and egg products fall under USDA. Everything else falls under FDA. Inspections of imported food by FDA are decreasing. Testing for food pathogens require large sample size (65-75g). Most testing focuses on Listeria, E.coli, Salmonella and STECs. There is a strong preference for validated methods and the methods should be easy and cheap. Detection is the main focus of innovation and innovation comes from clinical and bio-threat applications. However, sample preparation methods will not come from the clinical market since their samples are less complex than food and the level of pathogens in their samples are higher than in food, volume of samples are smaller, competitive flora not relevant, target flora are often known, pre-enrichment is required and high prices for testing are tolerated.

For food, need to ensure that the sample contains the analyteof interest. This is done via enrichment since bigger volume samples are not feasible. For the detection system, the sample is diluted and tested. The analyte can also be separated and concentrated affinity or physical methods such as centrifugation. Dilution is easy, cheap and effective but requires enrichment of the sample and compatibility with the dilution buffer. Affinity systems remove inhibitors and concentrate sample. These systems can be automated but use limited volumes and there is a cost element. Centrifugation is easy, efficient and concentrates the sample but the hardware can be costly and there may be target loss. Filtration concentrates the sample, accommodates large sample volumes but there are multiple steps and clogging is always a problem. Detection methods include culture, lateral flow devices, ELISA/ELFA, endpoint PCR, real-time PCR,

isothermal amplification and fully integrated systems. Real-time PCR is where the bulk of testing is occurring.. New approaches in all areas of food testing is needed.

On June 4, 2012, new regulations for STEC testing went into effect. ELISA and PCR methods are available. FSIS labs are using the published MLG method. Government wants low cost, easy, single test that uses a common enrichment. Individuals want methods that can take various sample sizes and include ground beef, produce and treated beef.

Food Pathogen Detection (T. Laruk, Strategic Diagnostics): Food testing is to provide consumers with safe food and reasonable cost. Economic drivers are production costs, food spoilage, avoid recalls and protect brands. The regulatory drivers are the Food Safety Modernization Act, HACCP, USDA FSIS, FDA, NPIP and international regulatory agencies. Standard practice is to test for food-related bacteria. Parties that are testing include suppliers (raw foods), food processors, routine testing labs and regulatory testing labs (USDA, FDA).

Analytical methods include culture vs rapid methods. All methods use enrichment of the samples. Confirmation steps are done by regulatory labs. Rapid methods are highly sensitive and specific as well as rapid, simple, convenient and low cost.

The challenges for analytical methods include time to results (reality is 8-48 hours), accuracy (as good as or better than reference methods), compliance (understanding new regulations and keeping operations compliant as well as verifying testing technology is accepted).

The rule for sample enrichment is “if you can’t grow it, you can’t show it”. The goal is to increase the target organism in the sample. The steps for enrichment are:

Media → mix → incubate → transfer to detection process

Detection methods include culture, PCR and immunoassay (agglutination, microwell, LFD, automated). For Listeria, 80% of the testing is done surfaces. The testing occurs before running production and right after sanitation. Zone 1 is meat cutting, zone 2 is exterior of equipment, zone 3 is walls, lifts, drains, zone 4 is outside. HACCP is also used. Listeeria grows readily at room temperature so the production area must be sanitized between shifts. The length of testing determines how the production is held in refrigeration. The impact of inaccurate methods is false positives and false negatives. False positives result in re-cleaning the facility, retesting. False negatives result in Listeria being in the product that goes to consumer which results in recalls (millions of dollars).

Mycotoxin Analysis in Grains Using LFD Test Kits, ELISA Test Kits, HPLC or LC-MS/MS Reference Methods (C. Brewe, Romer – given by D. Layton): Mycotoxins are naturally occurring toxic secondary metabolites of fungi. They have a diverse range of effects and are found on almost all agricultural commodities. More than 300 mycotoxins have been identified. Regulatory limits have been established in over 100 countries. Classes of mycotoxins include aflatoxins, trichothecenes and fumonisins from *Aspergillus* and *Fusaria.* Mild infection by the fungi can happen in the field or during storage of grain. The absence of the fungus (or mold) does not mean absence of the mycotoxin. Mycotoxins are not uniformly distributed in the crop.

Established action levels by countries determine the testing. Regulatory actions are taken against products that exceed the action level.. The guidance level is just a guide as to what is acceptable. The EU prohibits the use of products that do not comply with maximum levels.

The procedural flow for testing is:

Sample → grind → extract → purify → analyze

Sampling erro is the biggest component of the total error of analysis. Rapid tests include ELISA, LFDs, fluorometric. ELISA is a competitive format and is sensitive and rapid. LFDs are single sample tests and are usually qualitative, rapid and easy. There are many HPLC reference methods and AOAC official methods. LC-MS/MS is selective and sensitive, however, it requires expensive equipment and is expensive.

A New Method for Soybean Agglutinin Analysis (M. Breeze, Monsanto): The objective was to identify and evaluate methodology that increases the precision/quality of information developed on soybean agglutinin (SBA) levels. SBA is an anti-nutrient in soybean which is difficult to quantitate during GM product assessment. Lectins bind to specific sugar moieties based upon lectin type. Agglutination of lectins *in vitro* is the basis for analytical methods currently used. One hemagglutination unit is a measure of lectin content and is defined as the level that causes 50% of the blood cells in suspension to sediment in 2.5 hours. The assay units are arbitrary and the test lacks specificity. It is also time consuming and resource intensive as well as varying between labs. Bridging between techniques is not feasible due to red blood cell source and technique dependency.

Modern methods that might be useful include surface plasmon resonance, affinity chromatography, MALDI TOF. All of these require expensive instrumentation and trained personnel. ELISA increases precision, accuracy, specificity and robustness. Another method is ELLA (enzyme-linked lectin analysis). This method is activity-based and is based on binding kinetics. A hybrid ELISA/ELLA approach is a combination of published methods with the potential to change working range of either technique alone and improve performance.

The ELISA method is a standard double sandwich format which detects any active (tetramer) form and inactive (monomer) form of the protein. It is specific for SBA and uses the soybean lectin as the standard (from Sigma). The ELLA method uses multivalent monosaccharide-polyacrylamide conjugate and is dependent on tetrameric nature of SBA. The method would detect any lectin with galactose specificity similar to red blood cell hemagglutination kinetics. ELLA is commercially available.

The hybrid methods tested were: a) ELISA capture/ELLA detect or b) ELLA capture/ELISA detect. The benefits of the hybrid method is improvement of precision, express results in quantitative units (mg/g sample), have higher throughput than hemagglutination (48 samples/day), easily conduct with standard lab equipment and offers ability to define natural variability of SBA in soybean.

Method Comparison

	Red Blood Cells	ELLA	ELSIA/ELLA
CV	>20%		9%
Duration of test	2.5 hours	1 hour	2 hours
Range	0 – 512X range	1X	1X
Sensitivity	+/-	pg	ng

It was suggested that the hybrid method be submitted to AOCS and have reviewers look at it. Monsanto would like to publish their results when they are done, however, they would prefer to have publications from AEIC or AOCS, i.e., more powerful for an official method. The AEIC Board will take this up for consideration.

NEW MEMBER TALK

Douglas Scientific (D. Cook): Douglas Scientific is owned by Douglas ESOP. The company is located in Alexandria, Minnesota and is employee-owned. Douglas Machine, Inc. is involved in consumer products, automated package machines and was founded in 1964. Douglas Scientific was founded in 2009 and has 80 employees and sites in 14+ countries with over 100 instruments. Their vision is to help customers do it better, faster and cheaper.

Douglas Scientific has a disruptive array tape automation platform which is based on embossed tape with wells that use very small reaction volumes (1-2ul). The tape is continuous polymer media, essentially a roll of microwell plates. The instrumentation uses the array tape, Nexar in-line liquid handling, Soellex PCR waterbath, high capacity thermocycler and can handle 250,000 samples. Scanning is accomplished with Arraya in-line scanning.

Currently working with EnviroLogix to release DNAble endpoint assays. Douglas plans to release a quantitative nucleic acid platform in 2013 which will use the isothermal DNAble chemistry. It is a fully integrated assay setup.

Platform expansion is planned to include nanoQuad family of dispense solutions, Arraya 2.0 detection system, enhanced software solutions, sample preparation and purification solutions,multiplexing support/capability, immunoassay application solutions and collaborative development opportunities. Douglas is an emerging leader in lab automation. For more information: www.douglasscientific.com.

List of Fall Meeting 2012 Attendees

	Name	Affiliation
1	Penny Hunst	Bayer
2	Dean Layton	EnviroLogix
3	Clara Alarcon	DuPont Pioneer
4	Guomin Shan	Dow AgroSciences
5	Indira Ratnayaka	Canadian Grain Commission
6	Tom Currier	Bayer
7	Jean Schmidt	DuPont Pioneer
8	Jian Zhang	BASF
9	Joe Hudson	Bayer
10	Ryan Johnson	BioDiagnostics
11	Laura Privalle	BASF
12	Michele Yarnall	Syngenta
13	Gina Clapper	AOCS
14	Tandace Bell	USDA GIPSA
15	Jane Sabbatini	Covance
16	Matt Breeze	Monsanto
17	David Levin	Covance
18	John Jackson	Monsanto
19	Yelena Dudin	Monsanto
20	Suzanne Miller	Agri-Diagnostic Mfg.
21	Satish Rai	Douglas Scientific
22	Hope Hart	Syngenta
23	Darren Cook	Douglas Scientific
24	Padma Sudarshana	Monsanto Veg. Seeds
25	Angela Umthun (canceled)	Stine Seeds
26	Ryan Sizemore	Monsanto
27	Robert Bohannon	Agdia
28	Lucy Liu	Monsanto

29	Hans Miche	Douglas Scientific
30	Eric Ma	Dow AgroSciences
31	Denise Thiede	BioDiagnostics
32	Craig McLain	Douglas Scientific
33	Dave Rambow (canceled)	Agdia
34	Daniel DeMarco	DuPont Qualicon
35	Brian Skoczenski	Acadia BioScience
36	Sonja Van Holland	SGS
37	Terry Goddard	EnviroLogix
38	Martina Newell-McGloughlin	UC Davis
39	Rae Cote	Eurofins
40	Frank Spiegelhalter	Eurofins GeneScan
41	Joan Lawten	EnviroLogix
42	David Jones	Primera Analytical Solutions
43	Bibo Xu	Primera Analytical Solutions
44	Christy Brewe	Romer Labs
45	Venkatramana Pegadaraju	BioDiagnostics
46	Vikrant Dutta	EnviroLogix
47	Kwok Yeung	
48	Daniel DeMarco	Qualicon

TECHNICAL SERVICES AOCS

Save the Date!

2013 AOCS Technical Services Workshop: Laboratory Methods

July 16–17, 2013

FFA Enrichment Center, Des Moines Area Community College, Ankeny, IA

Organizer: Edward F. Askew, PhD, Askew Scientific Consulting

This inaugural AOCS Technical Services workshop includes educational sessions designed for lab technicians. An opening reception and two networking breaks will provide participants with an opportunity to reconnect with colleagues or meet new contacts.

Technical program topics include:

- Regulatory updates
- Method troubleshooting for food, feed, and biofuel
- Emerging pollutants
- Quality control and accreditation needs/requirements
- Publishing methods with AOCS

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For more details, visit
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