AEIC Business Meeting

Secretary Minutes of 2017 Spring Meeting: A motion was made, seconded and voted positive to approve the minutes of the 2017 Spring Meeting as posted on the website.

Treasurer Report (D. Layton):

<table>
<thead>
<tr>
<th></th>
<th>Planned ($)</th>
<th>Actual ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beginning balance Jan 2017</strong></td>
<td>12166</td>
<td>12166</td>
</tr>
<tr>
<td><strong>Dues</strong></td>
<td>9500</td>
<td>8700</td>
</tr>
<tr>
<td><strong>TOTAL Revenue</strong></td>
<td>9500</td>
<td>8700</td>
</tr>
<tr>
<td><strong>Expenses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scientific paper</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>DE Franchise report</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>ANSI/ISO (AOCS TAG)</td>
<td>2900</td>
<td>2900</td>
</tr>
<tr>
<td>Board meeting</td>
<td>800</td>
<td>451</td>
</tr>
<tr>
<td>Spring 2017 Meeting</td>
<td>2000</td>
<td>1071</td>
</tr>
<tr>
<td>Website maintenance</td>
<td>3000</td>
<td>1853</td>
</tr>
<tr>
<td>Credit card processing</td>
<td>600</td>
<td>310</td>
</tr>
<tr>
<td>Fall 2017 Meeting</td>
<td>4500</td>
<td>6144 (estimate)</td>
</tr>
<tr>
<td>Graphic design</td>
<td></td>
<td></td>
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<tr>
<td>Reprints</td>
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<td></td>
</tr>
<tr>
<td>Subscriptions</td>
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<td></td>
</tr>
<tr>
<td>Misc</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL Expenses</strong></td>
<td>16025</td>
<td>12755</td>
</tr>
<tr>
<td><strong>Projected Balance</strong></td>
<td>5641</td>
<td>8111</td>
</tr>
</tbody>
</table>

The new dues structure will be implemented in 2018 as well as the meeting registration fee ($50/person). The new dues structure is:
<table>
<thead>
<tr>
<th>Companies</th>
<th>Previous dues amount</th>
<th>New dues amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (&gt;1000 employees)</td>
<td>$500</td>
<td>$1000</td>
</tr>
<tr>
<td>Medium (&gt;50 to &lt;1000 employees)</td>
<td>$500</td>
<td></td>
</tr>
<tr>
<td>Small (&lt;50 employees)</td>
<td>$250</td>
<td>$250</td>
</tr>
<tr>
<td>Associate members</td>
<td>$50</td>
<td>$50</td>
</tr>
<tr>
<td>Individual members</td>
<td>$100</td>
<td>$100</td>
</tr>
</tbody>
</table>

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

**Membership update (D. Layton):** The membership is as follows:

<table>
<thead>
<tr>
<th>Company Size</th>
<th>Current Number</th>
<th>Potential dues ($)</th>
<th>Actual Number</th>
<th>Actual dues ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large ($1000)</td>
<td>9</td>
<td>9000</td>
<td>10</td>
<td>10000</td>
</tr>
<tr>
<td>Medium ($500)</td>
<td>13</td>
<td>6500</td>
<td>14</td>
<td>7000</td>
</tr>
<tr>
<td>Small ($250)</td>
<td>10</td>
<td>2500</td>
<td>10</td>
<td>2500</td>
</tr>
<tr>
<td>Associate ($50)</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Individual ($100)</td>
<td>4</td>
<td>400</td>
<td>4</td>
<td>400</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>38</strong></td>
<td><strong>18500</strong></td>
<td><strong>40</strong></td>
<td><strong>20000</strong></td>
</tr>
</tbody>
</table>

**Website (D. Layton, R. Shillito):** A subcommittee organized at the Spring Meeting found issues on the website and these were corrected. The website is in need of updated pictures and a request has gone out to membership. EnviroLogix indicated that they have an employee who may be able to assist in a redesign.

**AEIC Vice President Nominations (C. Pick):** Nomination were taken at the meeting for VP as follows:

Brenda Johnson (Eurofins Biodiagnostics), Susan Tapley (EnviroLogix), Donna Houchins (Romer). The Secretary will send out a message to membership for any further nominations. The Secretary will also confirm with the nominees their commitment to be on the ballot. Ballots will be sent out at the beginning of November.

**2018 Spring Meeting (C. Pick):** The meeting will be hosted by OMIC USA in Portland, Oregon. Suggested dates were April 24-25 or April 25-26. The membership suggested the following topics:

- Focus crop: Sunflowers
- Turfgrass talks. Suggested speakers were Scott’s, faculty of Oregon State, Dave Stimpson, Dan Curry
- Cannabis production and use: analysis needs
- Canola as a matrix and the issues it presents
- Seed health testing
- Varietal identification testing for rice (OMIC)
- Oil testing for DNA
Volunteers for hosting the 2018 Fall Meeting were sought. Eurofins GeneScan volunteered to have the meeting in New Orleans.

**Updates:**

**ISO/TC 34 TAG (R. Shillito, Bayer):** The ISO standards are used by labs. If companies/groups want input into these standards and how they are developed, participation in the TAG allows this. The following are examples of ISO Technical Committees (TC):

- TC 34: Food Products
- TC 47: Chemistry
- TC 48: Lab Equipment
- TC 69: Application of Statistical Methods
- TC 134: Fertilizers and Soil Conditioners

TC 34 update: Committee was able to counter the use of ISO standards to restrict trade and helped to enable trade. Committee also prevented the adoption of the unworkable sampling standard and influenced the way sampling methods are used. TC 34 also gained input into standards for PCR and protein methods and is developing a standard for the validation of qualitative methods. Participation in TC 34 affords members positive professional relationships.

The ISO Plenary Meeting was held in September 2017. Issues discussed were microarray analysis, varietal identification, qualitative method validation and operation of plant pathology labs. The ISO process is dominated mainly by one government. By joining a TAG, companies/groups can be active in developing the U.S. consensus on standards. TAG membership is open and members are expected to be active in the meetings/calls. Experts submit new ideas for standards. Conveners lead teams that write and edit the standard documents. The TAG members determine the member country position. The fixed TAG costs are $12000 to maintain the U.S. as chair. The USDA currently covers this cost. To maintain U.S. participation in SC 16 Biomolecular Testing is $2700 which is the cost that AEIC has been providing. The U.S. TAG chair is Ray Shillito. An associate chair is needed so volunteers are appreciated.

Examples of working groups (WG) are as follows:

- WG 3: Varietal Identification
- WG 4: Plant Pathogens
- WG 5: Qualitative Methods
- WG 8: Meat Speciation
- WG 9: Sub-sampling

**Cartegena Protocol (R. Shillito, Bayer):** The Cartegena Protocol (also known as Biosafety Protocol) is an agreement which deals with the biosafety of Living Modified Organisms (LMOs), their effects on biodiversity and any risks to human health. The last meeting was held in Cancun, Mexico in December 2016. The Global Industry Coalition is a network of labs which deals with detection method issues and issued a handbook. The Coalition is dealing with synthetic biology which is a hot topic.

**International Seed Trade Association (ISTA) (R. Shillito, Bayer):** ISTA has a GMO committee and proficiency program. ISTA also deals with certification of seed testing labs. The ISTA Statistical
Committee held a Seedcalc workshop in which Seedcalc will be converted to Bayesian statistics. ISTA has also held a seed purity symposium.

AACC Molecular Biomarkers for Grain Committee (R. Shillito, Bayer): The committee runs international workshops to train regulators and seed labs. Workshops have been held in 20 countries and most recently in Trinidad and Tobago. The Committee most recently offered public comments on labeling.

Composition Working Group (L. Muschinske, Covance): The CWG is a sub-committee of the AEIC which deals with the compositional analysis of crops to determine nutritional equivalence new crop products to those crops on the market. The CWG is looking at composition analysis methods that can be improved. Synopsis of methods being considered:

- The ELLA method (ELISA for lectins) is now an official AOCS method.
- Trypsin inhibitor analysis and whether it can be moved to a MS platform for streamlining and multiplexing.
- Until now fatty acids and CPFAs have been measured using two separate methods. Working to consolidate into one method to measure both.
- Consolidating methods for endogenous allergens to bring consistency and considering ring trials to test.
- Consolidate proximate methods to three approaches such as NIR.
- Combine analysis of several fat soluble vitamins into one method.

Breakout Sessions from 2017 Spring Meeting (R. Johnson, BASF): Ryan gave a follow-up on the breakout session topic from the last meeting on genome edited products and how to detect them. A discussion had occurred around detection of SNPs vs indels. SNP detection is not as easy as an indel. The group also felt that AEIC should be involved in government/authorities discussions on regulating such products and especially around the detection area. One suggestion was that AEIC could make a compilation of all the issues around detection of genome-edited products. The group also felt that AEIC should be more proactive rather than let other groups jump ahead with their suggestions/demands with the regulatory authorities.

Breakout Sessions from 2017 Fall Meeting: Two sessions were held: 1) Protein characterization and analysis and 2) Seed testing horizons and constraints.

Report from Protein Characterization session (M. Cheever, Bayer US): Discussion was around issues/challenges of post-translation modifications, digestibility, intractable proteins, multiplex platform validations, how to drive toward regulator acceptance of other technologies. A sub-group will be established to meet at the next AEIC meeting to discuss further.

Report from Seed Testing Horizons/Constraints (B. Johnson, Eurofins BDI): Seed testing companies would like access to whole seed controls for methods and suggested maybe AEIC could be involved in establishing a seed bank and sell samples. Discussion also occurred on whether bioassays should be replaced as the price point between PCR and bioassays is getting close. A challenge may be obtaining licensing agreements to allow the use of specific PCR assays for traits.

Showing of movie “Food Evolution” to attendees: The movie dealt with the polarized debate, marked by fear and distrust, concerning the controversy surrounding GMOs and food. The movie was narrated by
Invited Talks

Welcome to Indianapolis: The AEIC was welcomed to Indianapolis by Mark Krieger, representing the Ag Division of DowDuPont. Dow and DuPont merged as of August 31 and within 18 months will split into three companies: agriculture, materials science and specialty products. The agriculture division will be the current largest agriculture company for chemicals and seeds. Within the next 12 years, the division expects to launch 27 new products.

Tray Seed Tests (A. Patin, SGS): The purpose of seed testing is to determine the expressed percent of the presence or absence of herbicide tolerance in a seedlot. The tray tests are use to show individual plant expression and/or a screen to show the absence of herbicide tolerance in seedlot. The concentration of the herbicide used in the test must allow the seedlings to grow normally while non-tolerant seedlings do not. This is a challenge since lab and field herbicide concentrations do not correlate.

There are three methods for tray tests: seed soak, spray over and substrate imbibition. In the seed soak method, the seed is soaked in herbicide and then allowed to grow out. For the spray over test, the seed is grown out and then sprayed with the herbicide. And for substrate imbibition, the herbicide is in the medium that seeds grow out on. For glyphosate tolerant soybeans, the imbibition test is used since the herbicide absorbs into the foliage. The seedlings are allowed to grow for 7 days after imbibition (herbicide in versapak paper) and are then evaluated for germination and herbicide tolerance. For STS soybeans, the imbibition method (versapak) is used, but the versapak is covered with sand. The seed is allowed to grow out and the seedlings are assessed. For glufosinate tolerance, soybean seedlings are tested in the spray over method. Corn tray tests for glyphosate, glufosinate and STS are all done via imbibition assays. If a seedling is determined to be abnormal in a test, it is assessed as to whether this is due to mechanical injury, physiological decay, fungal infestation or the effect of the herbicide. Check samples are always verified. If the test is within 1.5% of the threshold, an additional 400 seeds are tested.

Tray and rolled towel methods (J. Milcarek, Indiana Crop Improvement): Tray tests are done to confirm the trait and the % of the seedling expressing the trait. The test is conducted using 200 seeds/tray, two replications. The herbicide is applied via versapak and incubated with the seed for 6 days. The seed is then evaluated for dead/abnormal seeds, short colorless roots (sign of non-herbicide tolerant). The percent normal seed is determined by subtracting the non-tolerant number from the normal number and dividing by the normal number times 100.

Spray over tests are conducted by growing seedlings for 5 days and then spraying with the herbicide of interest. Two hundred seeds are used as the sample. The seedlings are evaluated 5 days after spraying for normal, abnormal, not-tolerant.
For the rolled towel test, the germination paper is soaked with the herbicide and then 100 seeds (4 replications) are placed on the paper. The paper is rolled up with the seeds and placed in a planting basket. The seedlings are evaluated after 6 days.

**Soybean seed spray over test (R. Shillito, Bayer US):** For LibertyLink soybean, licensees must show 98% of 400 seed sample is tolerant to the herbicide. Glufosinate tolerant seedlings show no effect of the herbicide. Non-tolerant seedlings exhibit obvious browning, drying, death of cotyledons and unifoliate leaves. Licensees must pass a proficiency program and can use any method they choose. A test for IFT herbicide tolerance is coming soon.

**Canola and wheat testing (S. Foster, 20/20 Seed Labs):** 20/20 Seed Labs started testing herbicide tolerance in 1996. Bulk of testing is done on the herbicides glufosinate, glyphosate and imazamox. Recognized sampling methods are used to take samples and attention is given to eliminate any cross-contamination. The lab adheres to pure seed rules and uses controlled growth environments and train analysts. Herbicide dilutions are used which reflect the field rates. Typically 400-750 seed samples in multiple replications are used. Spray over testing is done at the 2-4 leaf stage in a spray chamber. The seedling are evaluated after 7-10 days. Wheat testing is done for Clearfield using the envelope method (paper folded over). The seedlings are evaluated after 10 days.

**Use of lateral flow strips (LFS) in cotton purity testing (M. Rowden, Bayer US):** A pneumatic loader is used to place seed in crushing trays which are then placed into a milled crush block. The seeds are crushed twice to ensure crushing of the embryo. The crushing blocks are washed with methanol and water to remove all tissue to prevent cross-contamination. The crushed embryo is agitated for 10 minutes in buffer and then a trait LFS is placed into each cell on the plate and the strips are then read as positive or negative.

**ELISA for qualitative/quantitative analysis (B. Johnson, Eurofins Biodiagnostics):** Trait providers require trait purity/expression testing according to licensing agreements. Agreements require that plant have insect resistance for best yield, plants have herbicide tolerance and ensure seed sold/purchased performs as expected.

For ELISA testing, seeds are germinated, tissue is harvested and protein is extracted. The ELISA test used should have specificity and normally is formatted as a sandwich assay: primary antibody bound to plate which captures the antigen. The captured antigen is detected by a secondary antibody to which an enzyme has been conjugated. When substrate is added, the enzyme facilitates a reaction which causes a color change. The results are read with a plate reader and then analyzed with an ELISA analysis tool. Results are reported to client.

**DNA-based purity testing (B. Rubin-Wilson, Dow AgroSciences):** Sources of impurity in seed production are segregation, stray pollen and admixtures. Purity testing is done for breeding work, regulatory and variety registration. An effective process for seed purity should have high throughput, be accurate, cost effective, rapid and synergize with other methods. DNA methods may be qualitative or quantitative, rapid, automatable/scalable, cost effective and environment neutral. The methods can be leveraged across crops and can be continuously improved.
Taqman PCR indicates whether the gene or event is present. It requires 30-60 min/plate and can be an endpoint or real-time assay. Endpoint assays are preferred for speed and throughput capability.

Endpoint PCR assays can be run in parallel with genetic purity. They can be optimized for use with crude lysate DNA extracts and run on super high-throughput automated platforms. They are biplex with endogenous gene controls. They do tend to be slower than other technologies and the costs are higher.

Zygosity testing is done to identify homozygotes and are performed with a single plant. Testing can be endpoint or real-time PCR. Single seed assays create a high workload and have a higher cost but the DNA can be share with genetic purity. Bulk seed assays allow more seed in fewer samples. DNA can be share with adventitious presence testing as part of some workflows. Bulk assays cannot distinguish between null and hemizygous seed. Bulk assays are superior to protein-based methods where transgene is dominant.

Isothermal amplification amplifies DNA at a single temperature. Method generally use a nicking enzyme and displacement. The method is not impacted crude lysate, is rapid, super high-throughput and can be deployed in the field. Costs are generally higher than other PCR methods.

Droplet digital PCR is a modification of conventional PCR which uses limiting dilution, endpoint PCR and Poisson statistics to yield direct measurement of DNA. It is independent of endogenous gene, effective in discriminating small differences and more accurate than other methods. However, it does have lower throughput than other methods.

NexGen sequencing has massive multi-dimensional multiplexing capability. It allows high coverage of relevant DNA regions and has the potential to combine trait purity, genetic purity, zygosity testing and adventitious presence testing. Its challenges are time to result and it may not align with high throughput.

**DNAble seed purity testing for biotech traits (S. Tapley, EnviroLogix):** DNAble is simple, instrument agnostic, rapid, isothermal nucleic acid amplification method with results similar to PCR. Crude samples are used with target specific primers and takes 25 minutes to complete the test. It is field portable testing for trait purity since minimal equipment is needed and is easy to use with no experience. Lyophilized master mix is resuspended and then sample is mixed in. It takes 7 minutes for the amplification. For adventitious presence testing, there is no DNA purification, sensitivity is down 0.1% and provides the ability to determine traits without DNA presence. High throughput method has been developed with the use of the IntelliQube platform.

**Cool seed grass production in the Willamette Valley of Oregon (B. Winsett, Hood River Seed):** There are lots of different species, both annual and perennial. Cool season grasses are produced in the following states/areas:
- Kentucky, Tennessee, Missouri, Arkansas > Kentucky-31 seed
- Northern Minnesota > timothy, brome, reed canarygrass
- Willamette Valley > fine fescues, tall fescues, perennial ryegrass, annual ryegrass, bluegrass, orchardgrass, bentgrass
The Willamette Valley of Oregon is 100 miles long and about 20-30 miles wide. It has deep soils and an ideal climate for grasses: wet, mild fall, winter and spring and a dry, mild summer. The area receives 40-50 inches of rainfall. Timing of rain is ideal (fall, winter) and usually stops in April growing season. There are about 1500 seed farmers. In the valley, grass seed production is 25% of the arable land (40% arable land). The rest of the land is used for forest (25%), urban (25%) and all other uses (25%). Grass seed is the 5th largest crop in Oregon and covers 400 acres in the valley. Approximately 600 million pounds of grass seed are produced with 98% of the seed going out of state. There is $375-400 million in direct sales every year and the industry employs 10,000 Oregonians. Grass seed accounts for $1 billion in economic activity through direct sales and support industries (ag equipment, fertilizer, chemicals, etc). About 600,000 tons of straw are exported to Asia each year. Screenings from seed cleaning are often pelletized and sold as a feed supplement.

Seed is thinly planted in rows and the rows are carbon banded. A layer of carbon is put over the seed rows. This allows the spraying of herbicides to control the volunteer seed. Seed is typically planted in the spring with harvest occurring the following year. Seed production is monitored closely for weeds, volunteer seedlings and disease. Usually there are 2-3 applications of fungicide made. Fields are swathed at 40-50% moisture. The swathed material is allowed to lay in field for 10-14 days to continue to ripen prior to final harvest. If the grass is swathed too early, this shortens the fill period. If it is swathed too late, this increases seed shattering. Most farmers have their own cleaning facilities and seed is cleaned on farm.

Grass production differs from grain production in that most grass is perennial and needs a vernalization period. Thus, it is grown in the same field for 3-10 years. This creates potential for genetic shifts. Most grasses are heterogenous populations so there is a spread in maturity within a variety. There are also numerous species/types within a species so it is important to guard against contamination. Other crops also compete for the land in the valley such as hops, filbert nuts, and grapes. Burning of the straw is essential for the stability of the grass crop. Burning eliminates the straw residue, insects, weeds and volunteer seedlings. However, open burning presents a health issue. In the 1980s, 250,000 acres were burned. By 2009, only 65,000 acres were burned and in 2017, only 15,000 acres were burned. Bentgrass needs field burning to produce seed.

Forage Genetics overview (D. Whalen and S. Temple, Forage Genetics): Forage Genetics (FGI) has done 25 years of alfalfa research and seed production and is currently conducting research on multiple forage crops. FGI has facilities across the country, all associated with dairy areas. Research capabilities exist in US, Argentina and Mexico. FGI develops conventional, organic and biotech seed. They are a member of Excellence Through Stewardship program.

All plants are genotyped prior to going into greenhouse crossing. This is a bottleneck in the process. FGI is working with industry and academics on coexistence strategies through the National Alfalfa and Forage Alliance (NAFA). NAFA is an umbrella organization of state/regional alfalfa seed/hay associations and their focus is issue advocacy and policy. It is governed by a 25 member board.

There are 20 million acres of forage spread over 48 states. Seed production occurs in 13 states on 120,000 acres. Alfalfa seed and hay can be exported. There are 6 grower opportunity zones (GOZ) in 8 states. A GOZ is a seed grower defined geography validated with a super majority of growers. No GMO seed production is allowed in the Imperial Valley, California, Canada or Australia. There is limited GM
seed production in 12 US states. Only 10% bloom is allowed in seed production areas. Bees are the major pollinators, with leaf cutter bees being the major pollinator. Honeybees do not do as well but are used in some states.

The seed production process includes breeder seed (Syn 1) which goes to produce stock seed (Syn 2) and then to commercial seed (Syn 3). Syn 3 is contracted with growers. Seed testing is done on 300-500 seeds/gram (there are 200,000 seeds/pound).

FGI has two biotech products deregulated by USDA: RRA (Roundup Ready Alfalfa; 2 events), HarvXtra (1 event). FGI also has food/feed approvals in key export markets. RRA is tolerant to glyphosate herbicides. HarvXtra alfalfa has had down regulation of the lignin pathway for increased digestibility by animals.

FGI has developed, proficiency tested and deployed DNA and protein assays. Event purity testing is done on leaf material. There is also zygosity testing and seed testing for green tag program. A platform for rapid DNA testing for RRA and HarvXtra has been developed and proficiency tested and is awaiting deployment.

**AgriPlex Genetics (A. Miron, AgriPlex):** AgriPlex Genetics was established in 2014 in Cleveland, OH. The company specializes in focused, simultaneous analysis of limited numbers of loci in any number of DNA samples in support of animal and plant breeders. The Next-Gen PlexSeq platform greatly improves throughput and efficiency while reducing costs.

The PlexSeq technology is focused next gen sequencing analysis for any SNP of interest. It is flexible to pick 10-3000 targets and has multiplex capabilities. It is an alternative to single plex and requires no ligation so there is no library preparation. It is cost effective, accurate genotyping solution. AgriPlex has analyzed 20676 samples simultaneously on single Illumna MiSeq run using 9 multiplex amplicons and obtaining 100 reads/SNP. The analysis software may be personalized to a specific project and specific SNP. 100 SNPs in 250,000 samples were analyzed resulting in 25 million genotypes in a week. AgriPlex has performed analyses on corn, soy and other crops.

**Application of seed coatings—conventional and biological (K. Gillis, Dow AgroSciences):** Seeds are coated to protect them from disease, pests, increase nutrient uptake and increase yield. Only focused chemical protection methods are available to date. Seed coating is complimentary to traits and provides grower ease of use.

There are batch treaters and continuous treaters. Batch treaters deliver a pre-determined batch size to mix chamber. Continuous treaters use weigh belt or seed wheel passes by atomizer to coat seeds. The chemicals used are specially formulated compounds and are a combination of oil and aqueous phases or suspended solids or blends of surfactants. Seed treatment products are a mix of ingredients and are costly. Polymers are also added to bind actives to seed. Treated seed must be colored with an unnatural color to indicate that it is treated seed.

Biologicales are rapidly growing segment and consist of living microbes, fermentation products or biologically-derived products. They have to be compatible with traditional chemical treatments and they must have resilience under mechanical stress of commercial equipment.
Treatment product preparations may be slurries or direct injection. Direct injection refers to directly injecting from the product containers into the seed treater. This provides increased flexibility for treating and less waste of chemicals. Dosing is done by bulk seed weight or seed size.

For batch treater, a seed quantity is precisely weighed and put into treater drum. The bottom of the drum rotates at a high speed and the treatment products are added onto a spinning disk. Seed treatment application may be affected by batch size, mix/dry time, drum speed, disk speed and baffle positioning.

In continuous flow treating, the seed flows into the treater at a controlled rate. Seed is directed around treatment impeller disk. The treatment products are added onto a spinning disk or aspirator which sprays out onto passing seed layer at outer edge of treater inlet.

Variability in treatment may be caused by seedlot quality, treatment slurry quality, application of slurry, sampling of treated seed and testing methods/labs. The quality criteria for treated seed include seed safety/germination, loading rate of treatment with label specifications, seed to seed distribution, flowability, dustiness of treated seeds, plantability of seed and durability of seed coating.

**Detection and identification of seed coatings on seed (A. Patin, SGS):** Seeds are coated with micro nutrients, nematocides, fungicides, insecticides, colorants/dyes, coating polymers, biologicals and organic seed treatments. It is important to confirm that a customer obtains the correct amount of treatment on the seed. Seed application equipment should be checked to ensure it is free of treatment contamination and it is properly calibrated. During the treatment, the physical appearance of the seed (tackiness, seed flow, visible dust off, appearance) should be monitored and the application rate should be verified. Test runs of the treatment recipe should be performed in small scale treatment equipment. Methods that are used to determine the amount of product on the seed include calibration verification of equipment, mass balance, ELISA, HPLC, culture plate for biologicals, GC and LC-MS.

Sampling of seed is done by bulk extraction of 50 seeds/extraction (large seeds use 50 seed replicates; small seed use two 10g replicates). The seed is agitated with the appropriate solvents and then filtered to remove large particles. Analytical methods such as HPLC are then employed to analyze the solvent extracted material. A series of reference standards are used in HPLC and calculations are done by regression analysis. Sources of variability include sampling, wrong HPLC column used, dilution issues and pipetting.

Similar methods are used to determine biologicals in coating. Biologicals include bacteria, fungi, plant extracts, micro or macro nutrients and peptides. Over-treatment is used to apply a second seed treatment recipe to previously treated seed. The compatibility of the treatment recipe should be verified and the total amount cannot exceed the maximum label rate. A one time application of all seed treatment products is the preferred process. For bacteria, determination of colony forming unit (cfu) is done. Bacteria are extracted from seed, put in a suspension, vortexed and then placed on a rocker. The preparation is diluted and plated out. The plates are incubated and then cfu counting is done (cfu/g). Errors may result from sampling, dilution, pipetting, extraction, plate media choice, uniformity of treatment and analyst morphology training. The cfu method does not measure efficacy.
**Remington Seed Tour:** A brief introduction to Remington Seed was given prior to participants going to the tour.

**Meeting was adjourned at noon.**

**Meeting Participants:**
20/20 Seed Labs  
Agdia  
AgReliant Genetics  
AgriPlex Genomics  
AOCS  
BASF  
Bayer U.S.  
Covance  
Dow AgroSciences  
DuPont Pioneer  
EnviroLogix  
Eurofins GeneScan  
Eurofins BioDiagnostics  
Eurofins Nutritional Analysis Center  
Forage Genetics Inc.  
Illinois Crop Improvement  
Indiana Crop Improvement  
LGC Genomics  
Merieux NutriSciences  
Monsanto  
Romer Labs  
SeqID  
SGS North America  
Stine Biotechnology