AEIC Spring Meeting 2005
Minutes

P.L. Hunst, Secretary

The AEIC Spring Meeting 2005 was held in Gastonia, NC and was hosted by USDA AMS at their facility. Chuck Martin (USDA AMS) welcomed the group to Gastonia and gave a brief introduction of USDA AMS' role in establishing standards for biotech crops. The Gastonia lab was built specifically to assist the USDA in the enforcement of the Federal Seed Act and compiling check samples for industry use.

AEIC Business Meeting

Approval of AEIC Fall Meeting 2004 Minutes: Motion was made, seconded and approved by the membership to accept the minutes as written.

Treasurer's Report:

Balance (Jan. 1)		$26638	
Dues	+$4300		
Acct Interest	+$ 14		
Projected Revenue	+$8590		
Expenses (Projected)			-$14323
Expenses (Paid to date)			-$ 4867
Projected Balance		**$16037**	

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

Membership: The current membership is as follows—

Large Companies	14
Small Companies	12
Assoc. Members	3
Individual Members	0
Total	29

Name of Organization: It was discussed at the Fall 2004 meeting that we may to change the acronym for organization, i.e., change what the letters "AEIC" stand for. It was decided to offer a $100 prize to whomever comes up with best tag line using the letters. Suggestions will be considered at the Fall Meeting 2005.

Powerpoint Presentation: The presentation is now available on the AEIC website (www.aeicbiotech.org) under "White Papers/Training". The slides are available in three different formats—flash, acrobat and Powerpoint. The slides can be downloaded for use.

Website: The website is again due for an update, especially the general information about the organization. Members are asked to send any suggestions for updates to the Secretary. The Secretary will work with the webmaster to update the relevant sections.

Other Business:

ISO WG7 Meeting: The meeting is tentatively scheduled to be held in New Orleans on Aug. 8-10. The meeting organizers are looking for sponsorship for the meeting, i.e., sponsoring a coffee break, lunch, etc. The organizers need $10-15,000 total sponsorship money and would like AEIC to contribute.

AOCS 2006: Meeting will be held in St. Louis (April 30-May 3, 2006). This may be an opportunity for AEIC to have exposure on an international level by participating in the "Hot Topics" session.

Fall 2005 Meeting: The meeting will be hosted by Agdia in Elkhart, IN. USDA GIPSA has offered to host the Spring 2006 meeting in Kansas City and EnviroLogix will host the Fall 2006 meeting in Portland, ME.

Suggested topics for the Fall 2005 meeting:

- Microarrays (Melcher from Oklahoma)
- Member company profiles (at least 2)
- Mycotoxins and transgenic plants (Iowa State)
- Unintended effects>metabolic profiling
- Mutagenesis products and how they are regulated
- Rhizobium-based expression system
- Customized allergen testing (Eurofins)
- PMPs
- Rice biotech>rice genome; golden rice
- Transient expression systems
- BioLex>expression in Lemnea
- Non-protein products/transcription factors/over-expressed endogenous proteins
- Method validations from medical or other areas
- Risk assessment for biotech>how is it done?

Presentations

Impact of the Biosafety Protocol on the Need for Biotechnology Methods (R. Giroux)
BSP impacts exporters and importers of grain, oilseeds, pulses and special crops--basically all crops intended for use as food, feed and further processing. This basically encompasses most crops that are traded. Each signatory to the BSP must develop a framework for a regulatory compliance. The crops impacted by the BSP include wheat, rice, barley, soybeans, corn, cotton, canola. Of the signatory countries to the BSP, the following characterizes the % of those that have ratified the BSP and are exporters/importers of the crop:

wheat	55% ratified
grain	56% ratified
corn	59% ratified
rice	46% ratified

Japan is the largest importer of corn and is a signatory of the BSP.

Decisions that are to be made by countries are the documentation requirements, i.e., "may contain" vs. specific information on the label; compliance and enforcement mechanisms; liability; and legal mechanisms. 2005 is a big year for implementation--all GM crops fall under Article 18.2(a) which means adventitious presence rules will be formulated; decisions will be made as to what information exporters will need to disclose about shipments and what the importer will need to do when the grain arrives in a BSP country.

For AP, how will this be determined--by a purity threshold? What will be the allowable level of AP in a shipment? Labeling--rules apply to crops for which LMOs have been commercialized only?

For labels, is "may contain" sufficient? Should BSP labeling resolve the issuance of new shipments or could relevant information be provided in commercial invoices? Should exporters acquire relevant information on LMO content of export cargo before they can export it? How will the importer receive and use the information provided by the exporter?

The US grain industry has conducted a case study in an effort to determine what the impact of these decisions will be. The case study considered the broad adoption of biotech traits in the US and that most of the production of these crops currently occurs in the US. Of the corn grown in the US, 25% goes to wet mills for processing into feed, HFCS, starches. Some also goes to ethanol production which is on the increase. Exported grain comprises 20% of US production. The majority of grain produced is used within the US for feed. The case study considered the outcomes of 3 potential labeling schemes: "contains LMOs", "identifies LMOs", "quantifies LMOs". The impact of these labeling schemes on the cost of testing shipments is projected as follows:

	"may contain"	"identifies LMOs"	"quantifies LMOs"
1 sample/cargo tested	$937,000	$2.3 million	$4.35 million
20 samples/cargo tested (EU)	$18.7 million	$46 million	$87 million

Who pays for testing? Importers pay, exporters do not. Exporting countries (US, CAN, etc.) do not have rules for BSP and most of the corn is used within these countries. Importing countries have compliance costs for the BSP and these costs are always passed on to the customers due to the costs incurred in selling. Large import volume countries (Japan, Korea, Mexico, China [has not yet ratified the BSP]) will likely incur large share of compliance costs. Developing countries will fare the worst since they will incur disproportionately higher costs due to the significantly smaller volumes imported. Costs are determined based on large volumes.

Summary of National Biotechnology Testing Methods (R. Shillito/M. Lipp)
Seed companies, government agencies, enforcement arms of agencies, grain handlers and food companies are a few of the groups who want detection methods. Seed companies test for purity and quality of transgenic and non-transgenic seed lots. Grain handlers and food/feed companies want to test for adventitious presence of unauthorized events and adventitious presence of authorized events (labeling regulations). They also want to be able to identify authorized events for traceability regulations. Government agencies and enforcement labs want tests in order to determine compliance to regulations.

Regulations demanding testing for transgenes.

	AP unauthorized events		Threshold authorized events
	Food	**Feed**	**Food Labeling**
US	0%	0%	None
Japan	0%	1%*	5%
Korea	0%	?	3%
China	0%	?	0.9% or 5% (TBD)
Taiwan	0/0.5%**	0/0.5%**	0.9%
Canada	0%	0%	None
Thailand	0%	0%	5%
Russia	0%	?	0.9%
South Africa	0%	0%	1%
Australia	0%	0%	1%
Saudi Arabia	0%	0%	1% (if approved in exporting country)

*If authorized in another OECD country.

**0.5% for events with positive safety evaluation; temporary measure.

There are many different thresholds for seed labeling. Here are few examples:

- Switzerland: 0.5% provision for recognition of approvals in other countries
- Argentina: Corn 1%
- Brazil: 1% in seed (cotton) of events registered in another country
- New Zealand: 0% (prescribed testing regime)
- USA: No specific requirements with regard to biotech
- Serbia: 0%
- EU proposal: GM varieties must be labeled as such; thresholds for non-GM varieties: 0.3 – 5% (cross-pollinating) 0.5 – 7% (self-pollinating) number in general category varies with crop kind and must approved for planting, otherwise it is 0%
- Italy: 0% (legal dispute)
- The above pertains only to authorized events; unauthorized events have a 0% tolerance in all countries

There are many methods out there, but they fall into 3 main categories: DNA-based, protein, trait-based/seed germination. For DNA-based methods, they come from national methods (Japan/Korea/China/others), private companies, biotechnology trait providers. There have been 53 methods submitted to ISO and/or Codex (which include methods for taxon, screening, events/constructs and proteins). The events covered by these methods are Bt11, CBH351, Event 176, GA21, MON810, T25 (all maize events); soybean GTS40-3-2; potato RBMT15-101, SEMT15-02, SEMT15-15; tomato Ailsa Craig Nema 282F.

The detection industry is organized into 4 major groups: internal corporate labs, commercial tesing labs, government testing labs and commercial test kit development companies. The internal corporate labs are mainly in large seed companies but are few are also in food companies. Commercial testing labs and government testing labs focus on seed, food and feed testing. Commercial test kit development companies primarily have focused on proteins tests, however, several do have PCR tests.

The current challenges of the testing include: a) lack of regulatory clarity and harmonization of testing criteria; b) lack of officially validated testing methods; c) lack of certified reference

materials and proper working standards; and d) lack of interaction with technology developers. Why is global harmonization for detection methods needed? Currently, methods are validated in different countries using different test items which may result in inconsistency of results throughout the value chain. Harmonization would eliminate errors in methodology and allow for orderly regulatory compliance throughout the value chain. There would also be uniformity of units of measurement and reduction in validation costs and duplication of efforts.

International validations over multiple continents are proceeding very slowly. Countries and trading blocks (Japan and EU) are doing local validations. In the EU, validation real-time PCR was started as part of 1829/2003 registrations. Five methods have been validated. Biotechnology providers have submitted pre-validated real-time quantitative PCR methods for many different events.

Methods scheduled for or completed (bold text) validation.

Crop	***Methods***
Corn	MON810, **Bt11, NK603, GA21**, **Mon863**, T25, **1507**, NK603xMON810, MON863xMON810, Bt176, GA21xMON810, NK603xMON863, MON810xMON863xNK603, 1507xNK603, 59122, MIR604
Rice	LLRICE62
Canola	Ms8, Rf, Rf2, Ms1, Topas 19/2, T45, GT73, Ms1xRf1, Ms1xRf2, Ms8xRf3
Cotton	MON1445, MON531, MON531xMON1445, MON15985xMON1445, MON15985, LL25
Sugar Beet	RUR H7
Potato	EH92-527-1
Soy	40-3-2

Laboratories using the methods are preferably accredited under official accreditation schemes such as ISO (17025), GLP, national schemes (AFNOR in France, UKAS in UK, DAP/DAR in Germany, CNAL in China). ISO accredited labs must validate official ISO methods for use in their own lab and have to participate in proficiency tests.

Reference materials and working standards come from public and private sources. IRMM (EU) is officially recognized by EU authorities as the producer of certified reference materials for GMOs and other materials. AOCS (US) produces certified reference materials for cotton (powder) and canola (seed) for Monsanto. Asahi Breweries and Nippon Flour Mills (Japan) produce plasmid working standards in collaboration with NFRI. The NRC (Canada) is exploring production schemes for canola reference materials. USDA GIPSA and NIST are not producing reference materials at present.

There are several issues that need to be resolved. These include sampling plans, extraction methods, effects of processing, multiple methods for the same target, endogenous genes and how to quickly test new approaches.

AEIC may want to consider several possible roles. These include capacity building (training in detection methods and slide sets for self-led training); standards setting (acceptability criteria);

harmonization of methods (adoption of standard methods, submission of methods to ISO, etc., setting up validations, endogenous gene harmonization, quickly testing new approaches).

Microsatellite analysis for compliance purposes (M. Sussman, USDA AMS)
Microsatellite work started as an urgent request from Florida where inspectors needed to distinguish two varieties. A microsatellite is a stretch of DNA with mono, di-, tri- or tetranucleotide units repeated. The dinucleotide repeated unit is the most common. Microsatellites are referred to as SSR (single sequence repeats), STR (short tandem repeats) and VNTR (variable number tandem repeats). The number of repeats can vary between species, varieties and individuals. Sequences flanking repeats are normally unique.

Microsatellites are useful because a) they are hypervariable due to a high mutation rate; b) segregate with chromosomes during mitosis; c) are randomly distributed throughout the genome; d) primer sequences are generally available from the literature; and e) they show exceptional variability.

An example of the use of microsatellites was given for avocadoes. Avocadoes must meet minimum standards, otherwise they are rejected. If they are picked too soon, they will not ripen for the customer. Avocado varieties look the same so they must be distinguished genetically. Use of microsatellites has been very successful as a deterrent to illegal imports.

Another example was the uglyripe tomato which is grown in Florida and Mexico. The uglyripe tomato is an heirloom tomato with homegrown flavor. Currently, uglyripe tomatoes cannot be shipped from Florida since they are not bred to be round (as per the minimum standards) and the FTC has insisted that the tomatoes fall under the minimum standards. The Senate has a solution in bill S.531 which would terminate the restrictions on the uglyripe tomatoes. USDA AMS is currently developing microsatellite methodology for enforcement for the uglyripe which will allow inspectors the ability to distinguish them from lower quality tomatoes.

Detection technologies and crop biosecurity (P. Berger – USDA APHIS PPQ)
Detection methods should have the desirable characteristics of low false negative/false positive rate, validated, easy to perform, high throughput and multi-dimensional. For crop biosecurity, there are a number of hosts, targets and tissue types. These include plant pathogens (nematodes, viruses, bacteria, fungi), invasive weeds, arthropods, snails/slugs.

Pathways for introduction of the agents are through intentional introduction (bioterror, smuggling), natural spread (soybean rust), tourism and hurricanes (citrus canker), trade (Asian longhorn beetle), trade or unknown (sudden oak death), and propagative material (plum pox virus). The challenges for detection of these pests include a) the diversity and numbers of crops/pests; b) 1 billion acres of crops grown domestically; c) a narrow knowledge base; d) response and recover; e) threat awareness; and f) technology ("state of the art").

The terrorism act has defined select agents which are the highest priorities. These included soybean rust (removed since it is already in US), plum pox virus (removed because it is not easy to work with). Only three of the top priority agents have immunoassay/PCR tests available and two of these agents have been removed from the list. There are no validated detection methods for the remaining agents.

To meet the challenges, several items are needed. These include: validated diagnostics, lab accreditation, harnessing of new technology, roles/responsibilities, information technologies and surge capacity (networks of labs). The commonly used diagnostics are ELISA (the workhorse),

molecular diagnostics (hybridization, PAGE, electron microscopy, PCR, micoarrays). In the future, diagnostics may include biosensors, gene fragment analysis, microarrays and multiplex real-time PCR (field deployable equipment).

The National Plant Diagnostic Network (NPDN) is under USDA CREES and has 5 hubs at universities located throughout the US. It is part of the plan (initiatives for offshore protection; emergency preparedness and response [ICS]; national plant disease recovery system) to safeguard US agriculture. Activities of APHIS PPQ include a national accreditation program for plant pathology labs. A workshop was held in Oct04 to gather information for such a program. It is estimated that it cost $6 million to start the program and $3 million/year to maintain it. Each lab must have appropriate facilities/infrastructure, be appropriately maintained and demonstrate proficiency. Assay validation is not well understood by plant pathologists or entomologists. Training is needed since they do not have the same tools (methodologies) to work with.

Implications of using multiple test methods in seeds (D. Dixon – Monsanto)
There are several different types of tests employed to manage seed inventory. Fixed attribute tests are applicable at breeding or trait integration phase. Commercial tests consist of in-line sampling vs. end point sampling. The sampling variability will impact the test results. Multiple samples/variety of test information provides more information on each seed lot. For instance, adventitious presence of biotech traits within a trait hybrid corn seed lot could be confirmed by PCR, ELISA and/or isozyme tests.

For pre-commercial seeds, trait purity is often determined by ELISA, field bioassay, herbicide rolled towel assay. Varietal purity is confirmed by DNA tests (SNPs, microsatellites, isozymes). For commercial seeds, tests must be conducted so that the seed lots will comply with regulations. These include viability, variety purity (must conform to standards), trait purity (comply with commercial contracts) and adventitious presence (may not exceed variety purity standards domestically; exported seed must comply with standards of the region exported to).

Adventitious presence policy within the seed industry may be defined by a specific numerical standard. The intent of the policy can be translated from words into specific rules for seed testing. The design of the tests and analytical approaches can impact.

Sample size drives the statistics and the analytical approach gives the best estimate of a sample. Most tolerances are based on the ISTA publication (Miles, 1963) and can be a comparison of a standard to an estimate or comparison of two estimates from the sample and/or the same lab. Seedcalc is an Excel-based statistical program with formulations to assist in designing appropriate test plans and managing risk. It is freely available at the ISTA website (www.seedtest.org) and applies to both qualitative and quantitative tests. Seed pooling can be used in qualitative tests to generate a quantitative test result.

In summary, it is valuable to test multiple attributes on a seed inventory. Qualitative testing of seed pools for adventitious presence of biotech traits provides appropriate qualitative management. Adventitious presence thresholds need to have clearly defined testing rules and official tolerances are needed.

Implications of using multiple seed testing methods in seeds: A test lab perspective (T. Gutormson, K. Brix-Davis – Mid-West Seed Services, Inc.)
Seed tests that are normally employed are the herbicide bioassay, ELISA (lateral flow strips or 96-well format), PCR (qualitative/quantitative) and electrophoresis. For the herbicide bioassay, 200-2000 seeds are tested. The herbicide is added to the media with trait and non-trait seedling

checks on each tray. The test evaluates normal seedlings for the trait and non-trait and the results are reported as % trait/normal seedlings. The rates used in the test are less than the field rates. The test is used for trait confirmation and adventitious presence. The limitations of the test include a 7 day turnaround time, seed quality interactions and the possible presence of dead/dormant seeds. However, the test does also give germination results.

ELISA tests (lateral flow strips) are quick and require minimal assets. Plate ELISA tests are used for determining purity on single seeds and they are easy, affordable and economical for testing %GM.

Qualitative PCR is used to quantify amount of a particular sequence and determine zygosity. There is a 3-4 day turnaround time and the limitations include no quantitation of GM, expensive and many sources of variability. Quantitative PCR is used for the quantification of the amount of a particular sequence and to determine zygosity. It also has a 3-4 day turnaround time, requires technical knowledge to conduct and is expensive.

Electrophoresis is used for isozyme analysis or total protein analysis. Pattern matches allow the determination of % of offtypes, selfs and purity. Electrophoresis cannot detect all differences, has a 3-10 day turnaround time and is dependent on the sampling method and extraction method.

It is important that the customer understands the uses and capabilities of each testing method.

Using multiple test methods in the supply chain: What is needed by industry (R. Giroux – Cargill)

Mandatory labeling is now enforced in 40 countries and this number is rising. Testing is used for determining the absence (unapproved events) or low level (approved events) biotech events. Another use is for the presence of GM events in seed, grain, ingredients and final foods. Testing may occur anywhere in the supply chain and depends upon the customer. Protein testing is generally conducted early in the supply chain and is relatively inexpensive. Testing later in the supply chain incurs costs that need to be passed on to the customer.

There are five needs of industry. Need 1: Consistent results for the product throughout the supply chain. Identity preserved chain test results must be consistent with regulatory compliance test results. Methods applied by third-party labs must be consistent to reduce risks of failure and test methods must be applicable to changes in material composition as it goes through the chain.

Need 2: Recognition that the testing marketplace for GM needs standards and standardization. The non-GM market is very risky since one bad test result with “sink the boat”. Governments need to recognize this is a global need and not just a national/regional issue. Methods need to be globally validated. Sources of method variability include sampling and quantitative testing. Sampling is the single source of testing error. Particle size will also affect in that if the particles are too large, it results in not being a representative sample. Quantitative testing is not simply the absence or presence but the variability around the mean must be considered. Larger samples produce more precise estimates but cost more to process. Precision is dependent on particle size.

Need 3: Recognition that sampling is critical. Extraction chemistry does matter and contributes to method variability. Reference materials used as method calibrants may give different analytical results such as the use of Event 176 (2 copies of 35s) vs. MON810 (1 copy of 35s). The presence of polysaccharides may affect PCR by inhibiting reactions, therefore, proper controls must be used.

Need 4: Recognition that all methods are not created equal. Processing for food (splitting the grain) will affect the ploidy since the germ is removed. This may affect the outcome of the PCR test.

Need 5: Recognition that screening methods for food are not accurate for food testing. It must be recognized that variability exists in a final test result for a finished food and act appropriately. Enforcement limits and policies that recognize imprecision should be implemented.

Transgene analysis methods and applications (D. Grothaus – Pioneer Hi-Bred)
Detection methods are used throughout the whole chain from discovery to commercialization. ELISA can be used across hybrid lines and developmental stages. An effort was made to eliminate the sources of variaibility. One source was the making of fresh standard protein versus using stabilized standard protein solutions. Stabilized standards appeared to have less variability. A correlation of expression levels to protein activity in plants could be made.

PCR is used for zygosity testing and Southern analysis and quantitative PCR are used for segregation determination and copy numbers. An effort was undertaken to compare ELISA with qualitative PCR with the objective to find the best ELISA method. The assumption was that the PCR results were always correct. Two proteins and two kits were used and the results assisted in the determination of which test was the more appropriate to be used.

UPDATES

USDA AMS: USDA AMS will host the ISO subcommittee for biomolecular testing in seed involvement. AMS is currently expanding its abilities to do more testing. The fee schedule will be changing in Oct05 and AMS will be 100% user fee supported.

USDA GIPSA: A China-US joint working group on biotech will be coming to GIPSA for a workshop on biotech. The group is interested in the proficiency program, samples and validation. The meeting will occur just prior to the Midwest AOAC meeting and would be an opportunity for AEIC to meet with them.

Representatives from USDA APHIS, USDA GIPSA, EPA and USDA FAS went to China to participate in a workshop on biotech safety management policies, safety assessment policies, safety assessment of Bt cotton, environmental risk assessment and real-time PCR. The Chinese attendees were important scientists and biotech policy makers from the National Biosafety Committee, Ministry of Agriculture, CDC, SEPA, etc. USDA APHIS and EPA presented case studies explaining the US approach to risk assessment, how the US has conducted specific environmental reviews for specific crops, how the US system verified data reliability (examples of how much data was necessary to reach conclusions about long-term effects) and how the US handles public concerns about safety and attacks from NGOs. In the future, there will be a comprehensive bi-lateral workshop on food safety assessment. The Chinese delegation will be training at USDA GIPSA.

An overview of the latest results from the GIPSA proficiency program was given and discussed. Since there was so much discussion, it was suggested that GIPSA give a presentation at the next AEIC meeting on the program results.

PCR Paper: The paper was published in AOAC International journal and a PDF has been distributed to the AEIC members. The committee is now working on a Nature Biotech publication.

AACC MON810 Validation: The validation was accomplished. There have been some re-writes of the method by the authors but the group hopes to finish by the Oct05 AACC meeting.

Japan Workshop: The workshop was held in Oct04 and was organized by ILSI Japan. The recommendations of the workshop were to move toward harmonization on an international level and that decisions should be grounded in science rather than politics. Presentations from this workshop are available on the workshop website.

OECD Seed Scheme: There is no special working group for GM which is the first time in 5 years that this has happened. A workshop will be held instead at the annual meeting in Paris in Sep05. OECD is looking for sponsors/speakers which may be an opportunity for AEIC.

Canadian Seed Seminar: This was held Feb. 3, 2005 and was a varietal identification workshop. It involved all stakeholders. The need for DNA and protein testing was recognized but it has not yet been figured out as to how to attain certification.

ILSI Harmonization Intiative: The initiative is testing/validation for NAFTA and involves education on methods for enforcement, i.e., what should they look like. Currently, the US, Canada and Mexico view GM grain as substantially equivalent so they see no need for testing. However, the rest of the world is harmonizing so the US, Canada and Mexico need to be aware. The initiative is to bring them together for discussions to share with each other and reach consensus. Authors will be brought together soon to write proposal and then it will be sent to the agencies.

ASTA: ASTA is involved with ISO. The seed industry relations group has also been asked to weigh in on the ISTA rule proposals. ASTA is also preparing a white paper on accreditations, i.e., what is needed to comply with different international standards.

ISTA: A workshop on GM testing with FAO will be held. The annual meeting is scheduled for the last week in April. A discussion of the rules chapter on cultivar and species testing will be held. ISTA GMO taskforce was started in 2001. Proficiency testing has been active and the intent is to publish the recent work on the ISTA website (www.seedtest.org).

Codex CCMAS: The Committee on Methods Analysis and Sampling has a criteria paper for biotech. There are tons of comments. The US scrubbed the document for "GMO" and Korea questioned the positive/negative for qualitative tests. The document will be re-written and re-submitted. The Committee will also continue working on methods for analysis harmonization and is currently dealing with how to resolve disputes.

ISO/TAG: The chair of TC34 is mulling over options for biomolecular seed testing and trying to determine whether there will be a separate subcommittee or taskforce. AFNOR (France) is upset and R. Cantrill is working on discussion points to present to them. Subcommittees 2 and 4 have no interest in running a seed testing regimen.

ISO/TAG/TC34: The main issue is the standard on sampling. The other standards are through the system.

Bt 10 PCR Test: J. Fagan (GeneticID) joined the meeting by phone to discuss GeneticID's test for Bt10. The method involves two primer sets plus a third set that targets a single copy maize gene. The first set of primers targets an internal junction sequence common to Bt 10 and 11 but

not found on any other maize event that is commercialized (primer called Bt1011). The second primer targets the junction between the transgenic insert and the flanking genomic sequences (primer called Bt11). When these primers are employed in the PCR test, the following outcomes are possible:

Bt1011	negative	Bt11	negative >> indicates a clear absence of both Bt 10 and Bt 11
Bt1011	positive	Bt11	negative >> indicates that Bt10 is present
Bt1011	positive	Bt11	positive >> this is an ambiguous result and real-time quantitative PCR would need to be performed on the primer sets.

The precision of quantitfication of real-time PCR is +/- 20% for each primer set, therefore, a significant difference would require that the Bt1011 signal be more than 40% stronger than the signal for the Bt11 primer. The method is still being validated.

AEIC Role Discussion:

A short discussion was held as to what AEIC should focus on. The following questions were thrown out for future discussion:

What should AEIC undertake?
Should we undertake an inter-laboratory comparison study using member companies' labs?
Should we make a list of methods, i.e., start with a list of methods for 35s? Can the methods be compared?
What is the next generation of quick testing methods?
Should we give recommendations on methods?

The meeting was adjourned at noon on April 14.