

**MINUTES FROM THE MEETINGS OF
AAFCO'S LABORATORY METHODS AND SERVICES COMMITTEE**

January 20, 2010; 8:00- 05:00

at the Crown Plaza, Redondo Beach, CA

Present were:

NAME	E-MAIL ADDRESS	ORGANIZATION
Aaron Price	aaron.price@inspection.gc.ca	CFIA
Alex MacDonald	BeeMac201@aol.com	Pharma Science
Amy Johnson	amyj@aocs.org	AOCS
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Vicki Siegel	vsiegel@purdue.edu	IN Dept of Ag
Yan Zhang	yzhang@ethanolresearch.com	Nat Corn to Ethanol Res Center

Nancy Thiex opened the meeting. After introductions the following issues were covered:

Approval of Agenda – The agenda was approved. (attached).

Approval of Minutes from the September Meeting –The Minutes from the meeting held on September 15, 2009 in Philadelphia, PA was approved. (attached)

Status of FDA Submissions – Thiex reported that submissions of drug methods were on track -

- Lasalocid has been accepted by FDA – Issue closed
- Oxytetracycline has been accepted by FDA – Issue closed
- Decoquinatone has been accepted by FDA – Issue closed
- Monensin, Narasin and Salinomycin - Data package submitted to FDA last week
- Sulfamethazine to be sent to FDA
- CTC - Tami Stolzenbach at Alpharma will be working on an HPLC method for quantifying OTC at formulated and residue levels.
- Tylosin – Tommy Phillips was not present and had not had time to work on this method since his update at the last meeting.

Drug Residues in DDGS – Linda Benjamin provided an update on FDA’s activities related to drug residues in Dried Distillers Grain (DDG). FDA issued an “enforcement discretion “ letter regarding the use of virginiamycin in 1993. Recent questions regarding the use of antibiotics in the production process for DDG reactivated the issue. FDA pulled 30-40 DDG samples from a variety of sources and used them for the validation of the recently published LC-MS/MS method. The result of this survey will NOT be published.

There are around 200 ethanol plants in the US. FDA is currently doing a survey pulling 40 domestic and 30 import samples. No estimated completion date for this study.

Benjamin reported that FDA is expecting companies interested in supporting the distillers with microbial control chemicals to file food additive petitions.

Method for Virginiamycin Residue in DDG – Alex MacDonald explained that virginiamycin (VM) consists of two major factors and several minor factors that interact in a synergistic fashion and therefore should be assayed using a biological activity based assay. VM is manufactured and distributed exclusively by Phibro. Phibro’s Ethanol Process lab in St. Paul, MN together with a commercial lab has validated a method that provides good recoveries in the range of 0.5-1.5ppm. It may be possible to drive the LOQ down to 0.2PPM. The resulting extracts are fairly stable. The method may be obtained from Phibro (QA@Phibro.com). (Presentation attached)

FDA and CFIA both use HPLC-MS/MS based methodologies focusing on the M1 factor.

The use of Ion Chromatography with Electro-Chemical Detection for Determination of Neomycin, Tetracyclines and Tylosin – Jay Gandhi presented a preliminary report showing neomycin data (identifying both the B and C component with a LOQ around 1PPB), tetracycline data (missing OTC in the chromatograms, estimated sensitivity around 1PPM) and tylosin data (can only see one peak representing one or both of the two components). He will continue work on this issue. As part of his study he found that CTC solutions were photosensitive. (Presentation attached)

Method for Melengesterol Acetate in Animal Feeds and Premixes – Ian Schuetz reported on SD Dept of Ag and R-Biopharm’s studies of the applicability of the Ridascreen Melengesterol acetate kit for quantifying the melengesterol acetate content in feeds and premixes. The kit was originally developed and validated for determining this drug in animal tissue. Normal feeding levels are around 0.5ppm. The importance of using a sufficiently large sample size in order to get a representative sample was stressed. Precision data covering a blank feed, a medicated feed and a premix looked good. (Presentation attached)

Amino Acid Related Studies – Yanhong Zhang and Amy Johnson reported on the status of the most recent

amino acid collaborative study. Of the 16 labs participating 6 labs had already turned in results and Zhang presented a summary. The rest of the results are expected in soon. Preliminary data indicated that the HPLC/Ion-exchange/post-column derivatization seemed to be the strongest method

Zhang also reported on her current work involving the use of microwave digestion for preparing hydrolysates for tryptophan determination by LC-MS/MS and HPLC-UV. It was suggested that she explore using BaCl₂ instead of NaOH. She will also be looking to determine what other amino acids can be successfully quantitated following this digestion procedure.

The correlation of the amino acid content in DDGS with the amino acid content in corn was also reported based on two recent studies. The correlation was good for most of the amino acids. More detailed information can be obtained from <http://www.valueadded.org/renewableEnergy/ethanol/ddgs/> and http://www.ddgs.umn.edu/profiles/us_profile_comparison_march_2009.pdf

Finally Dr. Zhang reported on the issues surrounding protein availability in swine and the use of furosine as an indicator of lysine availability. (Presentation attached)

Fatty Acid Collab Project – Nancy and Amy Johnson reported that the project was moving forward albeit very slowly. Samples have been received in Nancy's lab and are ready to be prepared and split except those to come via AOCS. Since the samples have been stored at room temperature their fatty acid profile may have changed – the consensus however was that they should still be suitable for the study. It was recommended to have a single lab run a quick fatty acid profile on one of the sample sets to double-check for abnormalities. Protocol is not ready yet but study is expected to start this spring. (Report received from Gina Clapper attached)

Starch Method Validation – Mary Beth Hall reported that the AOAC method had known shortcomings including the unavailability of one or more of the listed chemicals. Her method is based on the glucose oxidase method and is similar to AOAC 996.11. Maltulose formation is reduced by performing the hydrolysis at a slightly acidic pH. Mary plans to do a “pre validation” exercise involving potential collaborators to ensure that labs are sufficiently familiar with the method prior to initiating the study. She is currently looking for feedback as to the sample types that should be covered as part of the general study or as part of an expanded study (matrix extension study) involving only a few labs as well as feedback on the draft validation protocol (attached).

A draft of a fund raising letter asking donors to contribute toward AOAC's fee for validating methods was also covered. It was agreed that the letter was very good but needed additional editing.

Vitamin A – Nancy reported that she together with Michael Stevenson, Regina Wixon, Ken Riter, Jen Kraus and Mary Koestner may start working on updating the method for Vitamin A.

Vitamin D and E Project – This item was postponed to the Annual meeting as the presenter, Dr. Dale Hill, was not able to attend.

Method for determining low levels of sugars –

Lars Reimann presented the data provided by Waters. The presentation did not contain any findings as to the concentration of individual sugars in the samples provided

Ian Schuetz reported on R-Biopharm's results when using their test kits on the samples provided by Nancy. To cover the profile 3 different test kits were used on each sample. Duplication was good (less than 5% CV between duplicates. (Presentation attached)

Discussion followed how these data compared with those provided by Metrohm at the previous meeting. Based on a cursory comparison the data looked quite different. Lars Reimann will compile a comparison of the two data sets for use as a basis for further discussion. (attached)

Nomination of Candidates to Replace Tom Jensen – Nancy asked for nominations. It was agreed that the preferred candidate should be an enthusiastic person with good fundraising skills.

Method Needs Statements (MNS) – Aaron Price reported that the most updated versions of each method statement was posted on the AAFCO web site. A draft template for preparing such documents is attached. Revisions to this draft template will be reviewed at AAFCO’s 2010 Annual meeting

- Fructans – question on the needed LOQ in light of formulations containing added fructans at levels of 1 lb/ton. It was agreed to send the MNS out with the Minutes for a 14 day comment period followed by a vote.
- Total Metals – Nancy reported that Terrence Field had offered to become the project leader. The AOAC’s Food Contaminants group has already attempted to address this. Terrence’s first charge will be to review the current MNS draft.
- Bacitracin – Since bacitracin is a multi-component drug there is an issue with correlating HPLC profiles with activity that may prohibit the use of this method. However, it was agreed to include the MNS with the Minutes for a 14 day comment period followed by a vote.
- Fatty Acids – Gina Clapper to review and comment on the current version. After incorporating her comments (if any) the MNS will be distributed and voted on.
- Vitamin D – Thiex requested help with this. Dale Hill has offered to provide comments.
- Vitamin E – Thiex in the process of drafting needs statement. Need helps. Potential sources for help are Dale Hill, ADM and Neil Craft, Craft Technologies. .

It was agreed to remove roxarsone, phytase and pesticide residues form the list.

Method Prioritization Survey – Aaron reported that he had tweaked the survey used in 2008 for use in 2010. It was agreed to remove methods that already had current, complete MNS since they were already in the “pipeline”. The survey will be distributed with the meeting minutes to committee members, advisors, and attendees as well as industry and state contacts listed in the OP

Medicated Feed Mixer – Alex MacDonald reported on the separation issues experienced following the grinding of some medicated feeds and how Purdue’s machine shop had developed a mixer that seemed to be able to quickly make homogeneous powders of dried ground samples. Vickie Siegel would test the prototype with an estimated price tag around \$2,500. Schematics for the tumbler are attached.

Mycotoxin Prevalence – Siegel reported on the data collected by several states and compiled by Lu Wetzler. While there were many positive samples it was difficult to estimate the impact of the data because very few (if any) of the samples were associated with any history as to the tonnage they represented. Any one interested in being part of a working group focusing on restructuring the survey should contact Nancy.

Future Symposia – The possibility of using symposia as a way to publicize accomplishments were discussed. The following subject / estimated completion dates were covered:

- Amino Acids, 6 mos
- Starch, 18 mos
- Sugars, 18 mos Jay & Ian
- Fatty acids, 18 mos
- Minerals/Elements, 12 mos
- Antibiotics by IC, 12 mos
- Tylosin, 18 mos
- Vitamin A, 12 mos
- Annual Meeting, 2011 or Midwest AOAC (NE)
- Vitamin D & Vitamin E Method Needs Statement

Foodshield, LabDir and other related web/IT issues – Further discussion postponed to the next meeting in August.

Leader of Working Group studying the feasibility of an AACO sponsored Laboratory Accreditation Program – Further discussion postponed to the next meeting in August.

Respectfully submitted,

Lars Reimann

Eurofins Scientific, Inc.

Attachments:

1. Agenda January 2010
2. Meeting Minutes 091509
3. Phibro Virginiamycin presentation
4. Drug Residue Determination using IC-ECD
5. Melengesterol acetate determination using RidaScreen test kit
6. Draft of Starch Method Validation Protocol
7. Yanhong Zhang presentation
8. Clapper AOCS
9. Waters Sugar Summary
10. R-Biopharm Sugar Summary
11. Sugar data comparison
12. Draft of Method Needs Template
13. Fructan Method Needs Statement
14. Bacitracin Needs Statement
15. Purdue tumbler project
16. Method Needs Survey