

AAFCO LM&SC Fat Soluble Vitamins Working Group

Vitamin A Team & Vitamin E Team

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Denver August 2015

Round-Robin Study

- **Survey Vitamin A and E methods for possible collaborative study**
- **9 Samples chosen to cover a wide range**
- **11 Methods for Vitamin A (7 submitted data)**
- **8 Methods for Vitamin E (6 submitted data)**

Samples

Matrix	Vit A (kIU/kg)	Vit E (IU/kg)
Animal Feed	15	88
Concentrate	35	54
Vitamin Premix	4400	17500
Animal Feed	7	40
Concentrate	75	55
Mineral Premix	240	2059
Animal Feed	7	55
Animal Feed	20	110
Dry Pet Food	15	460

Sample Preparation

- **AAFCO Check Samples were not processed and simply relabeled**
- **Other samples were ground and riffled into 12 portions**

Statistical Analysis (ANOVA)

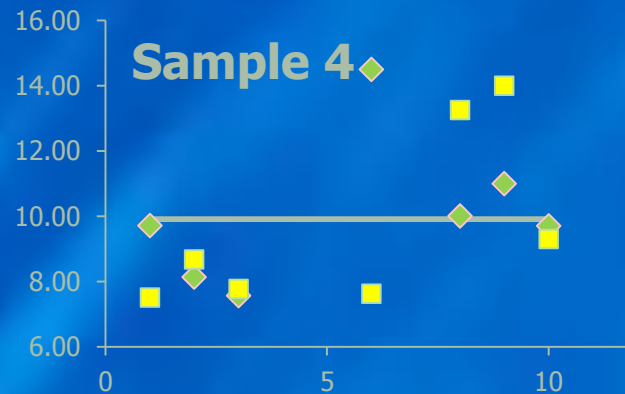
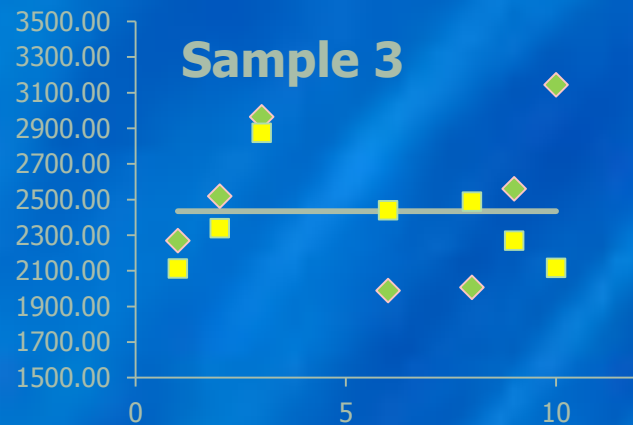
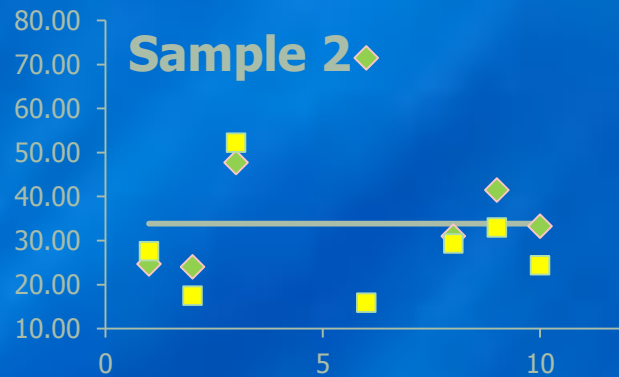
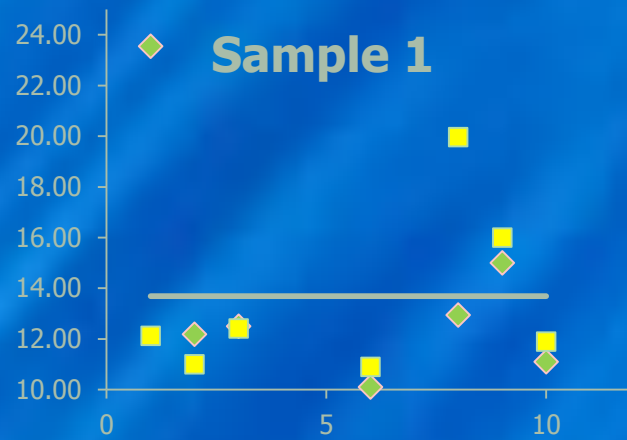
Vitamin A						
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Samples	70674235	7	10096319	685.625	0.000	2.178156
Methods	121910.8	6	20318.46	1.379794	0.239	2.265567
Interaction	724806.3	42	17257.29	1.171915	0.287	1.600222
Within	824640.1	56	14725.72			
Total	72345592	111				

Vitamin E						
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Samples	3.2E+09	8	4E+08	1024.966	0.000	2.115223
Methods	7435144	5	1487029	3.806821	0.005	2.38607
Interaction	56020402	40	1400510	3.585332	0.000	1.616172
Within	21093598	54	390622.2			
Total	3.29E+09	107				

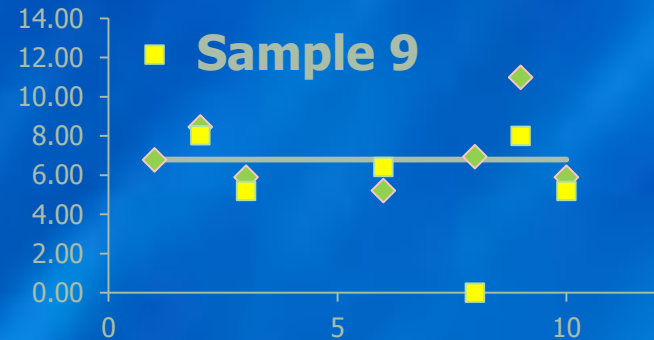
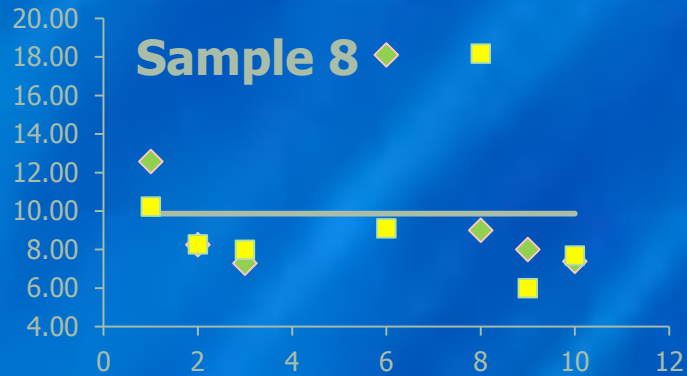
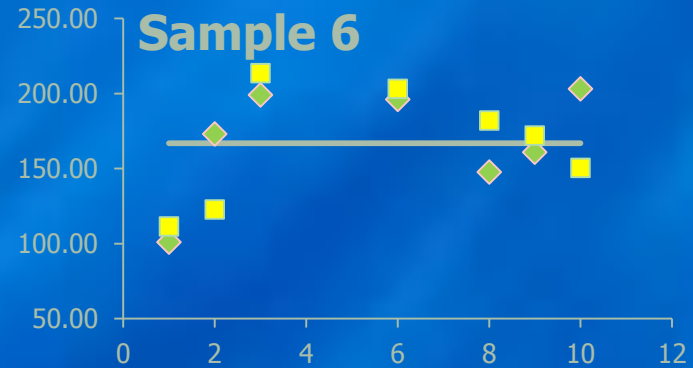
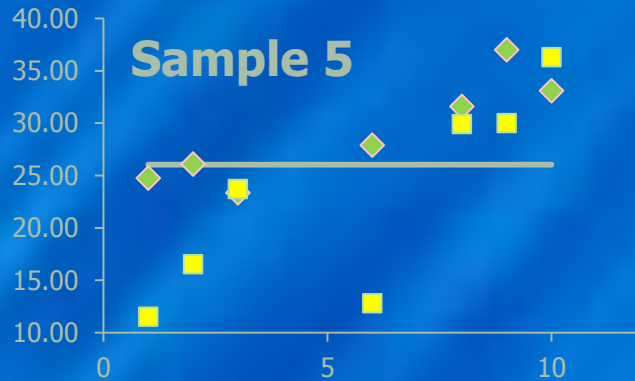
Statistical Analysis (ANOVA)

- **Vitamin A – No difference between methods**
- **Vitamin E – Method # 8 significantly lower than the others**

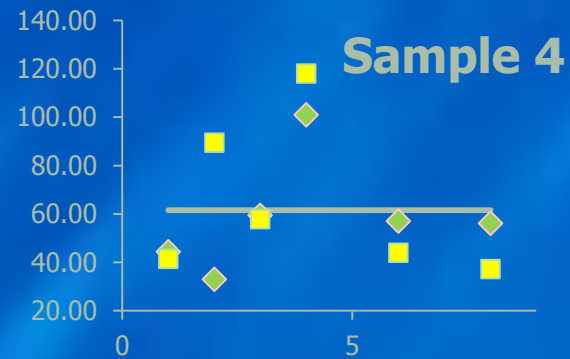
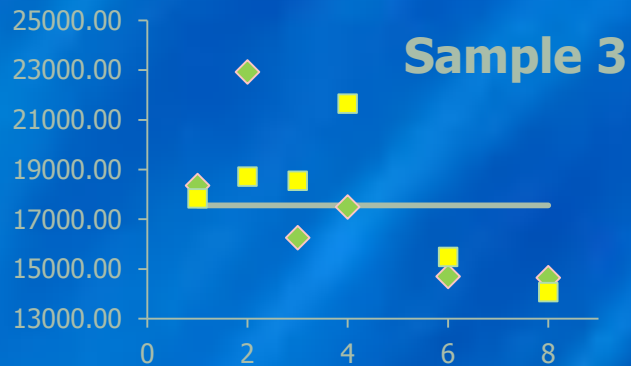
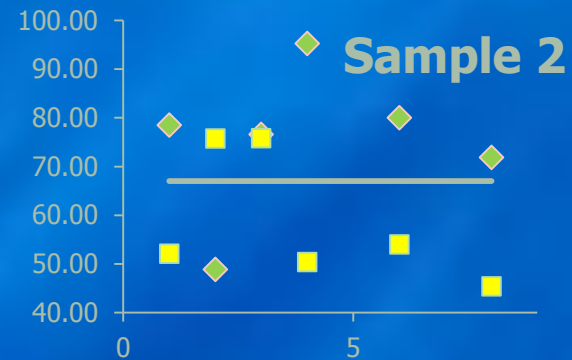
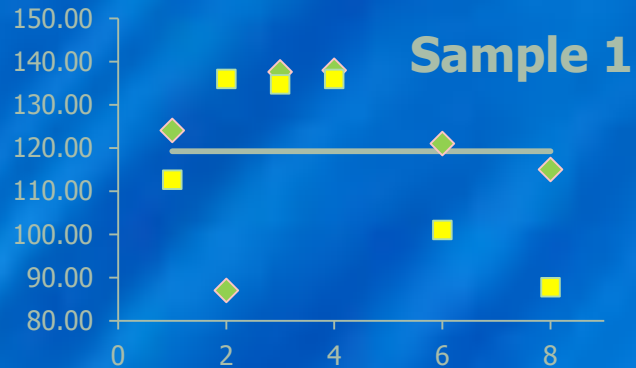
Vitamin A Data 1



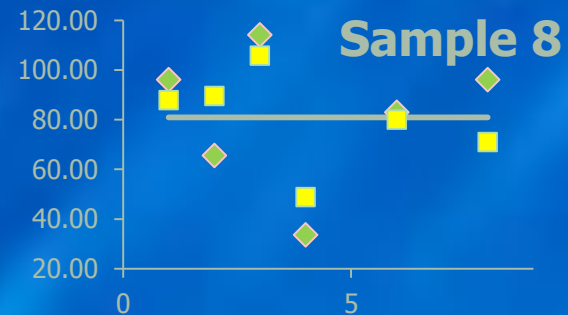
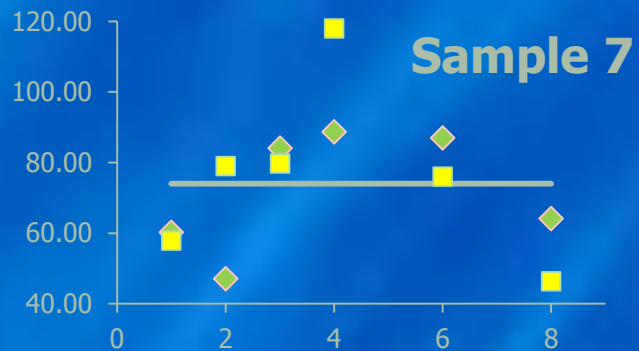
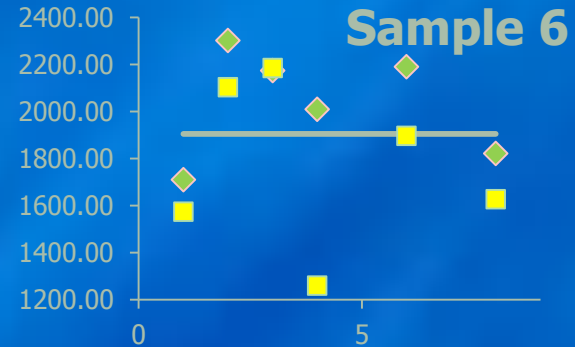
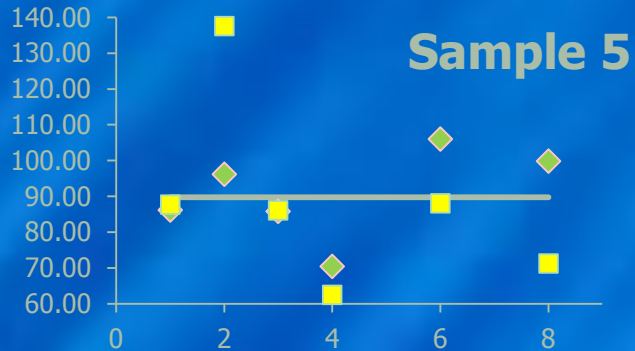
Vitamin A Data 2



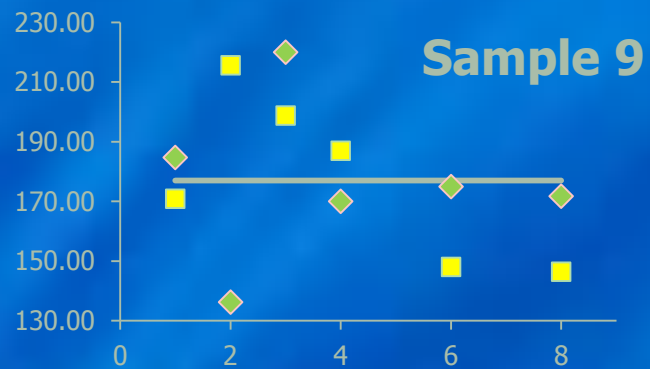
Vitamin E Data 1



Vitamin E Data 2



Vitamin E Data 3



How to Proceed?

- **Option 1**
 - ✓ Give Options for Extraction/Cleanup
 - ✓ Standardize HPLC or not
- **Option 2**
 - ✓ Select one method based on other factors (not results)
 - ✓ Examples: Ease-of-Use, sample throughput, multi-vitamin method, etc...

Action Items

- **Send your input or your interest in participating in the discussion to working group leaders**
 - ✓ H. Dorota Inerowicz – inerowic@purdue.edu
 - ✓ Ken Riter – ken.riter@purina.nestle.com
- **Discuss within working group and make recommendations to the committee**

Acknowledgments

- **ADM and the AAFCO Check Sample Committee for donating study samples**
- **Andy Crawford for providing statistical analysis of study data**
- **AAFCO Board of Directors for funding the working group's proposal**
- **Thanks to all of the laboratories participating in the study**