Aflatoxin Analysis: Implementation

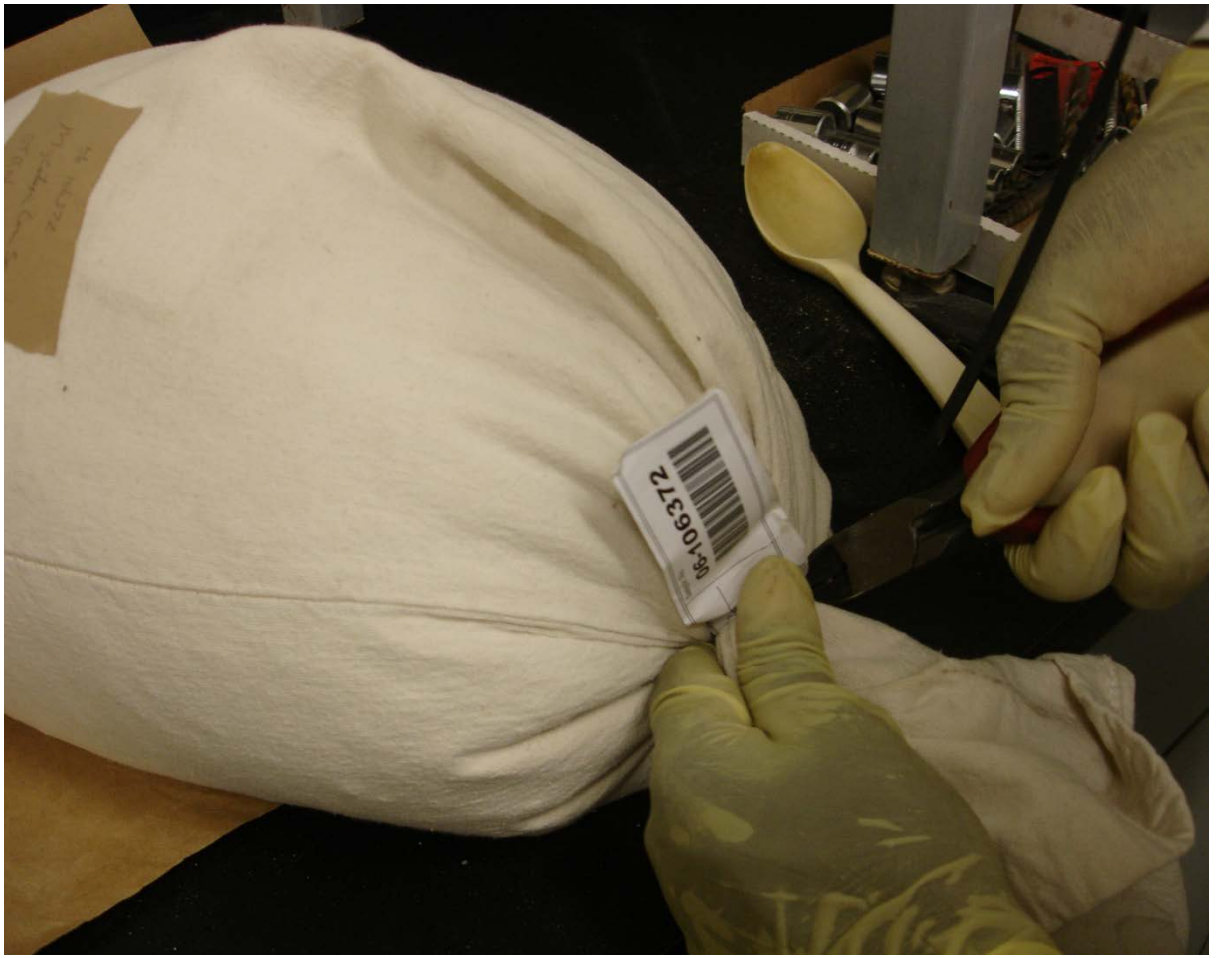
Sample Preparation

Standard Operating Procedures

Homogeneity

Stability

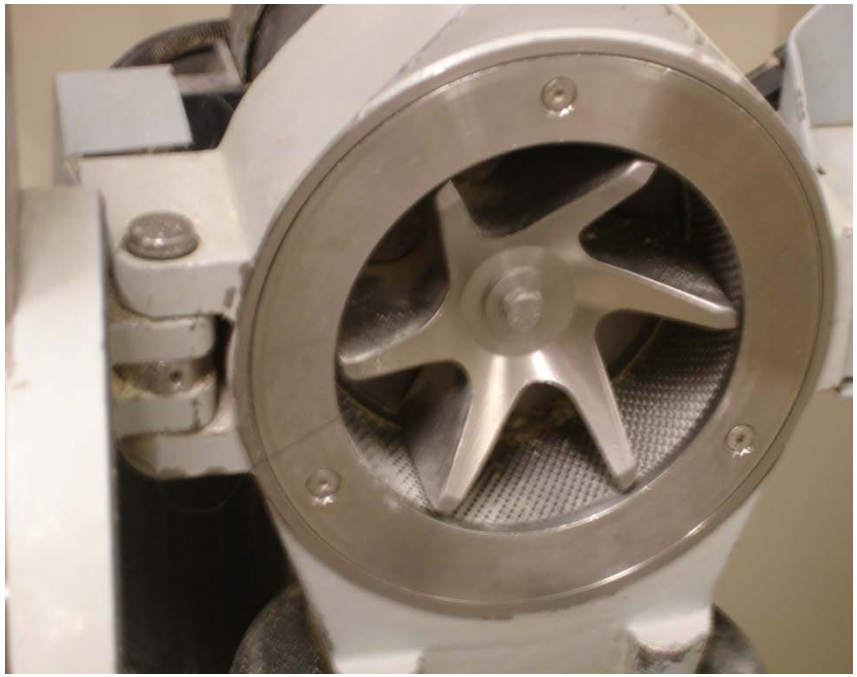
Chain of custody from field to lab



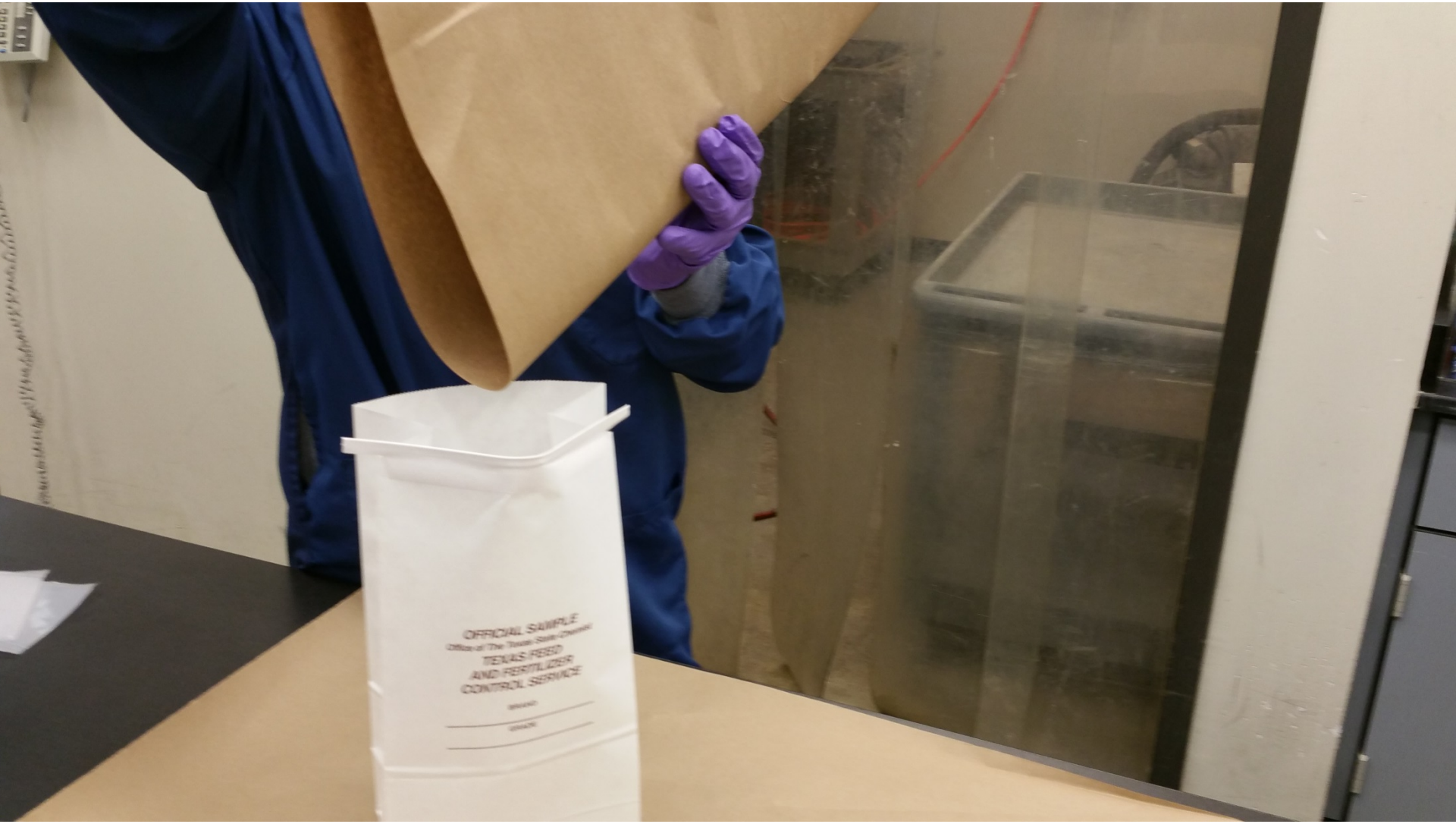
Sample Grinding

TEXAS A&M UNIVERSITY

Sample Grinding




Retsch

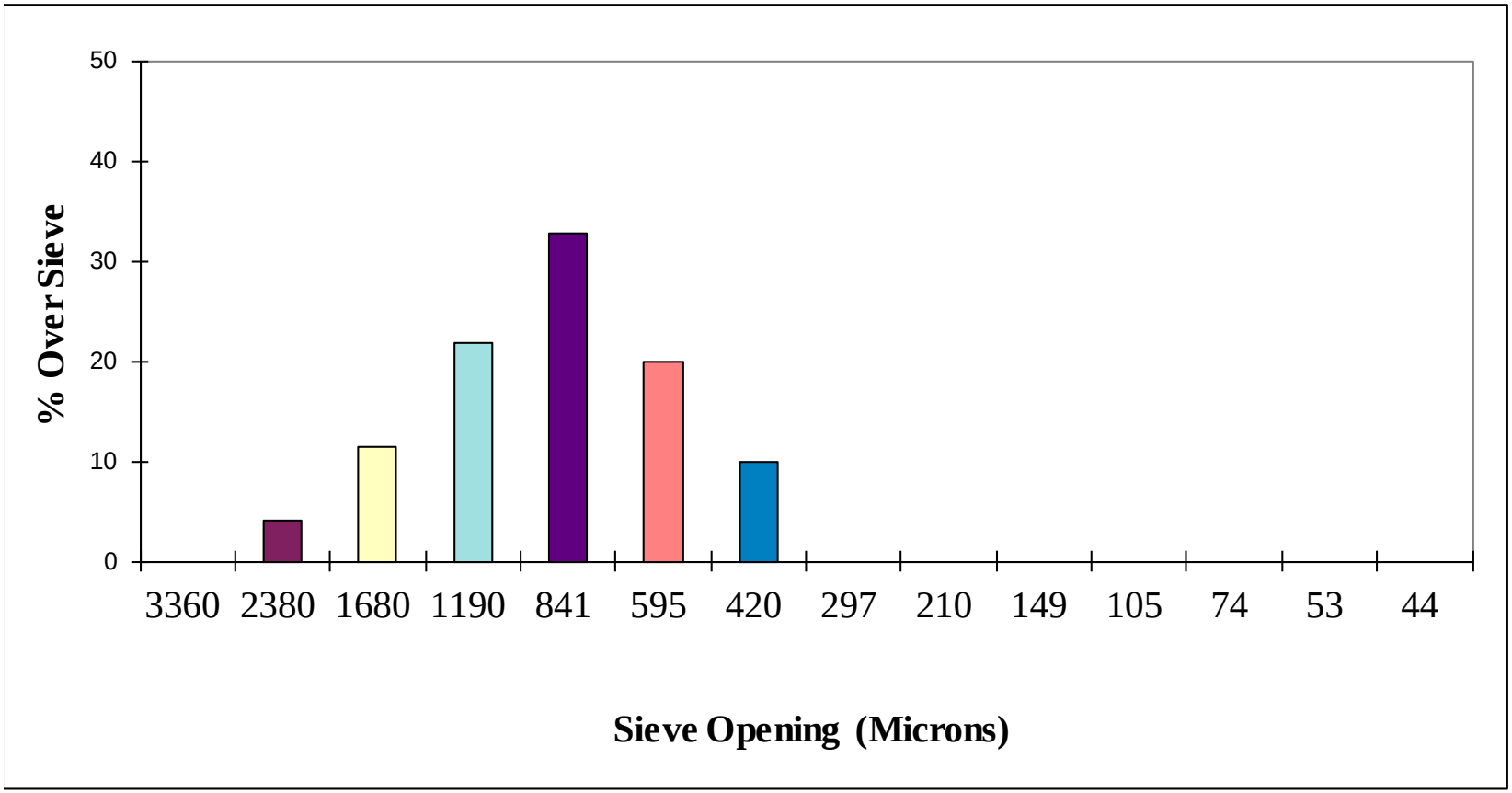

OFFICIAL SAMPLE
TEXAS FEED
AND FERTILIZER
CONTROL SERVICE

Criteria: Grinding

- Grind the entire sample
- Collect at least 500 grams of the ground sample
- 70% of the particles pass through a 20 mesh sieve after grinding

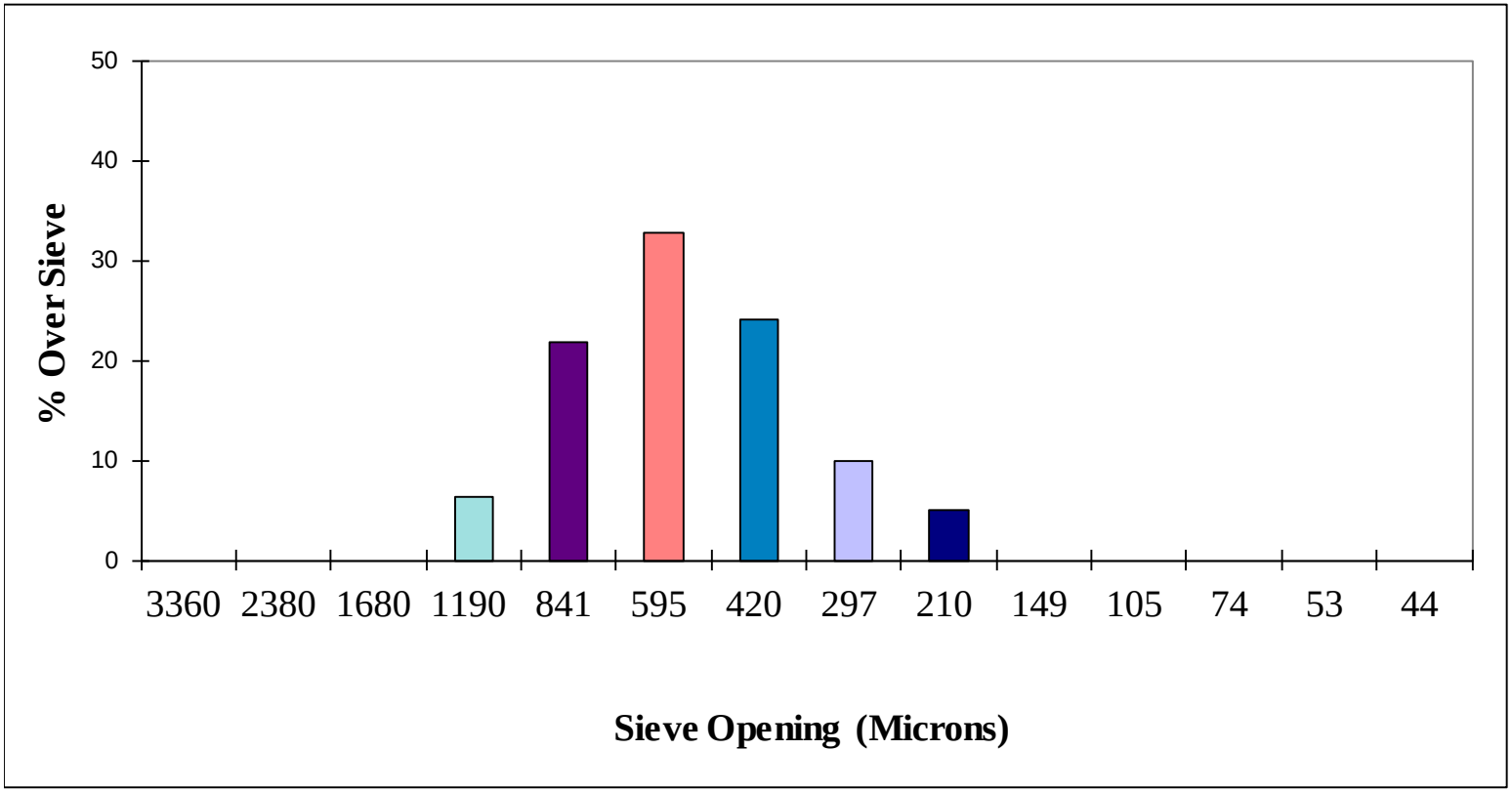
70% on top of 20 mesh sieve

Particle Size, Dgw	1059	Surface Area (cm^2) / gram	48.0
Standard Dev., Sgw	1.55	Particles / gram	1,541



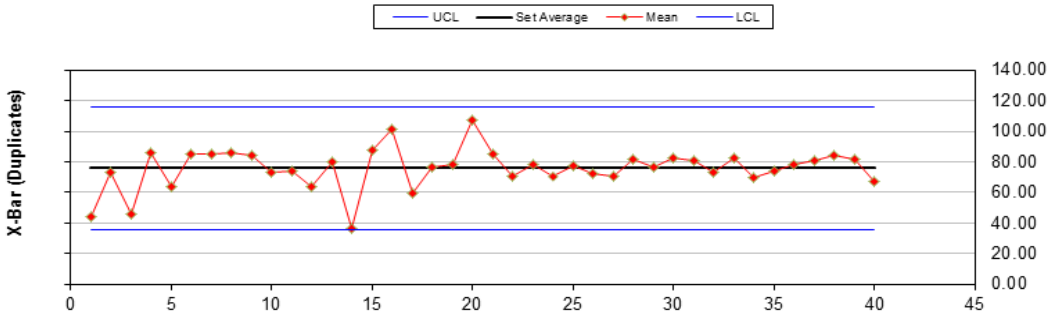
72% through a 20 mesh sieve

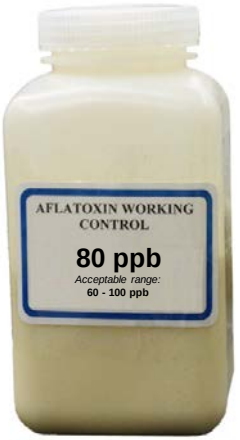
Particle Size, Dgw	650	Surface Area (cm^2) / gram	77.8
Standard Dev., Sgw	1.53	Particles / gram	6,333



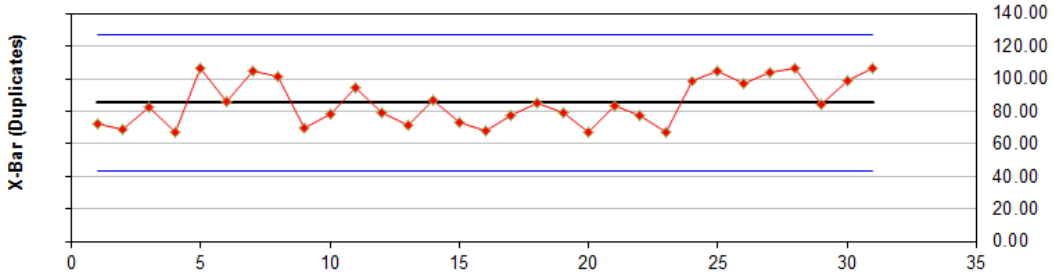
Control Chart

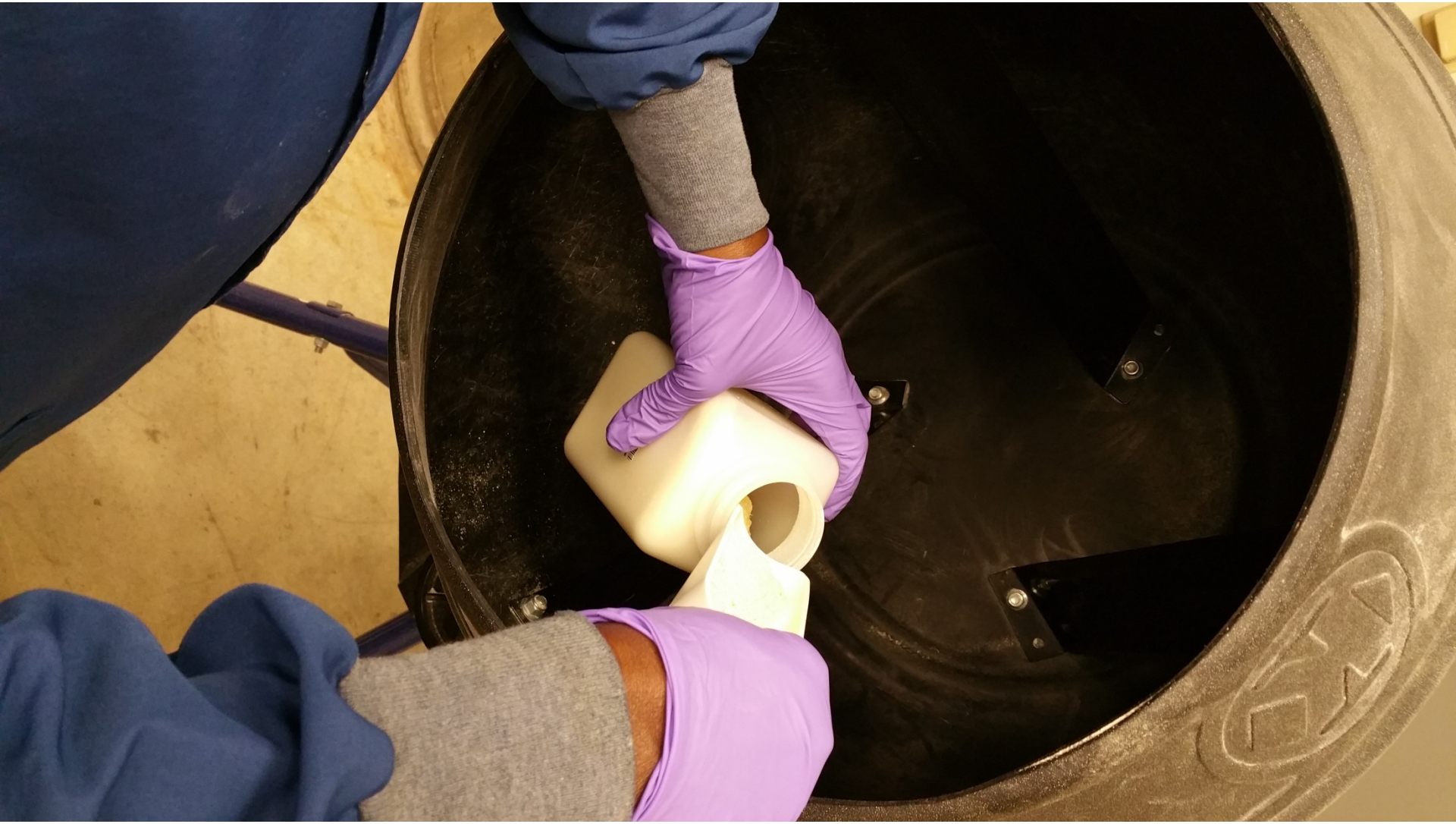
Company A



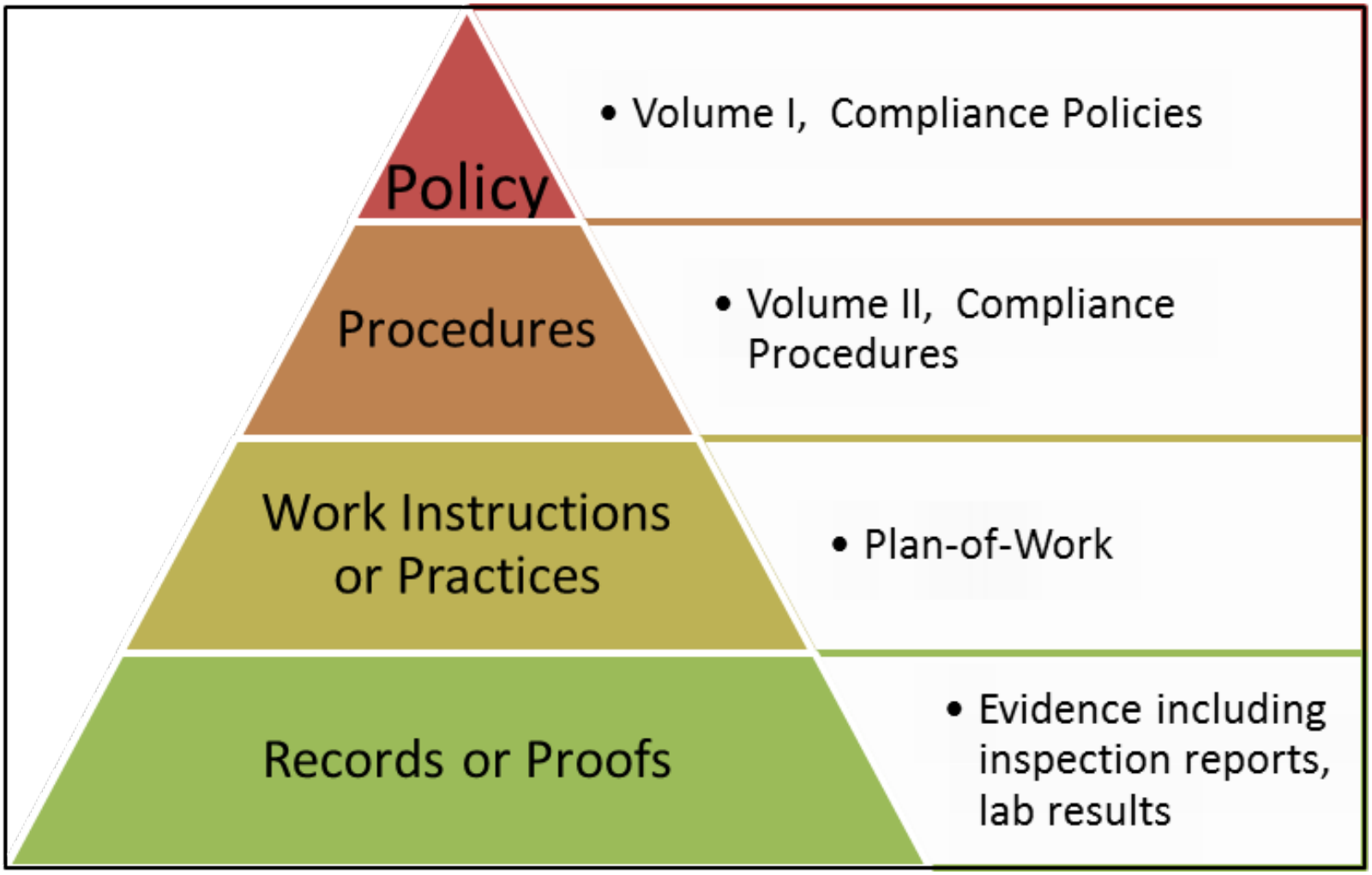


Company B





Standard Operating Procedures


Policy
• Volume I, Compliance Policies
Procedures
• Volume II, Compliance Procedures
Work Instructions or Practices
• Plan-of-Work
Records or Proofs
• Evidence including inspection reports, lab results

Why do we use SOPs

- Facilitate easier understanding of a procedure by the person being instructed
- Ensure more consistent training of multiple trainees by multiple trainers.
- Allow reviewers, auditors, and regulators to evaluate performance of a task in practice to the SOP
- Document the procedures to provide evidence of the consistency and completeness of the practice

OTSC SOP CONTENT

(Analytical Procedures)

Purpose

Scope/Field of Application

Definitions and Acronyms

Responsibilities

Materials Required

Procedure

Documentation

Reference Procedures

References

Revision History

M0053	*Quality System Procedure* **Office of the Texas State Chemist**	**Issue Date:**	**Rev.:** ***2***
Title:	**Control Standard Production for the One Sample Strategy Program**		Page #: ***1 of 3***

Purpose

To describe the process the laboratory takes in producing controls for the one sample aflatoxin program and for the aflatoxin in corn (maize) proficiency program.

Scope / Field of Application

This procedure will apply to One Sample Control Samples and Proficiency Test Items made from corn.

Responsibilities

Quality Manager assures SOP procedures and quality assurance procedures are followed.

Sample Prep Technician grinds, splits and packages corn samples

Chemist analyzes feed samples for aflatoxins

Materials Required

Plastic bottles, 1 liter, or any other suitable container

Romer Model R.A.S. Mill

Retsch SR300 Rotor Beater Mill

Screen, 1.5mm

Kobalt Mixer #0241568

Plastic heat sealable bags

20 mesh sieve

Procedure

Corn Acquisition (Two options)

1. Purchase - Purchase corn in quantities of 50 pounds per batch.

   When using this option, it would be wise to grind a test portion prior to preparing the entire batch to determine if the aflatoxin level is at the concentration required.

2. Combining - Mix previously analyzed samples together to make approximately a 50 pound batch.

   The expected concentration for a batch created using this option can be estimated by multiplying the weight by concentration for each sample. Sum this and then divide by the total weight.

Production of Test Item/Control Batch

1. Process the whole bag of corn with a R.A.S mill from Romer Lab.
2. Grind the corn through a commercial grinder (Retsch SR300) using a 1.5 mm screen.
3. Mix the ground corn for 1 hour with a commercial Kobalt mixer #0241568 so that it is thoroughly blended.

Packaging for One sample program

1. Scoop the ground corn into plastic bottles. Do not pack completely full. Leave some space at the top.

Packaging for proficiency program

2. Randomly selected bottles of the processed corn are poured into a bench top gray tub, a plastic spoon is used to alternately shovel portions of the grounded corn into plastic bags. Each spoon takes approximately 20 grams of the corn meal until the bag reaches the targeted weight (approximately 150 g). The plastic bag is then vacuum sealed and labeled for shipment.

Aflatoxin concentration determination

1. Sampling

   For each 50 pound bag, take 12 bottles of the prepared corn meal to determine the aflatoxin concentration. Weigh in duplicate 50 ± 0.25 g of the ground corn from the 12 bottles. The bottles should be randomly selected using the Excel RANDBETWEEN function.

2. Measurement method

   The assigned value for the aflatoxin concentration will be determined by an ISO accredited expert laboratory (OTSC-AAS) using a method (LC) on the laboratory's scope of accreditation. All twelve samples will be analyzed in duplicate in one batch on the same day. The twenty four analyses will be conducted in a random order generated using the EXCEL RANDBETWEEN function.

3. Evaluation of sample

   Use Appendix 1 of "The International harmonized protocol for the proficiency testing of analytical chemistry laboratories" to determine if the proficiency test item sample is sufficiently homogeneous.

Storage

1. Store each bottle of the proficiency test item sample in the -20 degree freezer until needed for distribution.
2. Use Appendix 2 of “The International harmonized protocol for the proficiency testing of analytical chemistry laboratories” to determine if the proficiency test item sample is altered during storage and/or transport. (A two-sample t-test will be conducted).
3. Five sample bottles will be analyzed before and after storage in the -20 freezer. Five proficiency samples in bags will also be analyzed post shipping.

References

ISO 17043 Conformity assessment-General requirements for proficiency testing

ISO 13528 Statistical methods for use in proficiency testing by interlaboratory comparisons

The International harmonized protocol for the proficiency testing of analytical chemistry laboratories Pure Appl. Chem. Vol 78 No.1 pp 145-196, 2006.

OTSC One Sample Strategy Handbook Version 5.0

SOP 16002 Aflatoxin in Feeds (Corn and Cottonseed Meal Products) by HPLC/PHRED

SOP 16004 Aflatoxin in Feeds by UHPLC/FLD

AAFCO Guidelines for Preparing Laboratory Samples 2008

Revision History

Major re-write to conform with ISO 17043 standard. November 2015

What is sufficient?

HOMOGENEITY

Sufficient Homogeneity

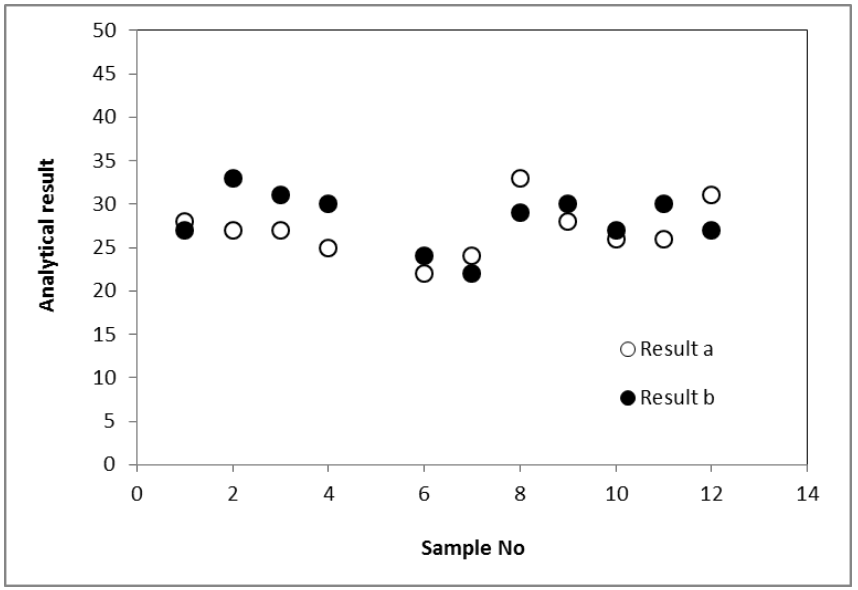
$$s_{sam} < \sigma_p$$

Estimated sampling standard deviation should be less than 30% of the target standard deviation σ_p

Criteria: 10 or more samples run in duplicate p170

Recommendation 9: Sufficient Homogeneity

In testing for sufficient homogeneity, duplicate results from a single distribution unit should be deleted before the analysis of variance if they are shown to be significantly different from each other by Cochran's test at the 99% level of confidence



Sample	Result a	Result b	D=a-b	S=a+b	$D^2 = (a - b)^2$
1	28	27	1.0	55.0	1.0
2	27	33	-6.0	60.0	36.0
3	27	31	-4.0	58.0	16.0
4	25	30	-5.0	55.0	25.0
6	22	24	-2.0	46.0	4.0
7	24	22	2.0	46.0	4.0
8	33	29	4.0	62.0	16.0
9	28	30	-2.0	58.0	4.0
10	26	27	-1.0	53.0	1.0
11	26	30	-4.0	56.0	16.0
12	31	27	4.0	58.0	16.0

Sum of squares ($S_{DD)}$	139	
Cochran's test	0.26	<0.57 (95%)
Analytical variance	6.32	(or ANOVA result)
Between sample variance (S = a + b)	26.76	
Between sample variance (Sampling variance)	3.53	Homogeneous
Target standard deviation (value of σ_p)	3.8	ppb
Allowable between-sample variance	1.2996	
Critical value [c]	14.19	

What is sufficient?

STABILITY

Sufficient stability

Changes in test material are inconsequential

Period in question is the interval between preparation of the material and the deadline for return of the results

5 samples will be analyzed after the proficiency test

Summary

- OTSC is transitioning from consensus statistics to an assigned value and Horwitz function to calculate Z-scores
- OTSC is adopting the harmonized protocol for proficiency testing as outlined in SOP M0053 to test for homogeneity and stability
- Particle size is important in sample preparation
- Reference material (or working controls) should be used to ensure accurate testing in addition to participating in proficiency tests

Aflatoxin Analysis: Performance Criteria

Test kit Validation

Analyst Qualification

Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits

1. Time required for completion of an analysis
2. Comparative accuracy of test kits on corn samples naturally contaminated with aflatoxin
3. Suggested additional commodities
4. Avoidance of toxic or hazardous substances
5. Sensitivity to electromagnetic fields
6. Temperature sensitivity
7. Stability
8. GIPSA Performance Verification

Test Materials

- Homegeneous
- Concentration range (5 ppb, 10 ppb, 20 ppb, 100 ppb)
- 95% of the material passes through a 20-mesh sieve
- 95% within the acceptable range

Acceptable limits

Concentration (ppb)	Maximum RSD_i (%)	Standard Deviation	Range* (ppb)
5.0	25	1.25	2.5 – 7.5
10	22	2.2	5.6 – 14.4
20	20	4.0	12 – 28
100	16	16	68 – 132

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Rapid Test Kit Validation by OTSC

Kit	Matrix	Toxin	Extraction	GIPSA Status	OTSC Result	OTSC Validation
Neogen Reveal Q+	Maize	Fumonsin	Organic	Approved	26% Accuracy	Fail
Charm Rose Wet	Maize	Fumonisin	Water	Approved	20% RSD	Fail
Charm Rose Fast	Maize	Fumonisin	Organic	Approved	30% Accuracy	Fail
Vicam	Maize	Fumonisin	Organic	Not approved	20% RSD -56% Acc	Fail
Charm Rosa Wet-S5	Maize	Aflatoxin	Water	Approved	RSD 22%	Fail
Vica Afla-V	Maize	Aflatoxin	Organic	Approved	37% RSD -40% Acc	Fail
Romer AgriStrip	Maize	Aflatoxin	Water	Approved	13% RSD 8% Acc	Pass

HPLC			
Average (ppb)	**Sample CV Average (%)**	**Duplication limit (%)**	**Result**
≤ 25	13.1	± 40	√
> 25 to ≤ 50	10.8	± 34	√
> 50 to ≤ 100	9.4	± 25	√
> 100	6.0	± 20	√

LC-MS			
Average (ppb)	**Sample CV Average (%)**	**Duplication limit (%)**	**Result**
≤ 25	18.4	± 40	√
> 25 to ≤ 50	15.3	± 34	√
> 50 to ≤ 100	11.9	± 25	√
> 100	9.5	± 20	√

ELISA			
Average (ppb)	**Sample CV Average (%)**	**Duplication limit (%)**	**Result**
≤ 25	12.4	± 40	√
> 25 to ≤ 50	16.1	± 34	√
> 50 to ≤ 100	14.7	± 25	√
> 100	9.9	± 20	√

TLC			
Average (ppb)	**Sample CV Average (%)**	**Duplication limit (%)**	**Result**
≤ 25	9.5	± 40	√
> 25 to ≤ 50	5.9	± 34	√
> 50 to ≤ 100	14.2	± 25	√
> 100	6.5	± 20	√

Incidence Passing (%)				
Sample Average (ppb)	**HPLC**	**LC-MS**	**ELISA**	**TLC**
≤ 25	97%	85%	100%	100%
> 25 to ≤ 50	96%	93%	91%	100%
> 50 to ≤ 100	93%	92%	83%	85%
> 100	98%	93%	93%	100%

the LC-MS had more samples that fell outside the range of the duplication limit at the lowest sample average (≤25 ppb) when compared to the other three testing platforms. Once at the higher concentrations, however, all platforms were much closer in how they met the duplication limit.

Who is qualified?

ANALYST QUALIFICATION

Procedure

GATHER INFORMATION:

The first step for qualifying in a new analysis is to read the SOP. A copy of this document can be obtained from the laboratory manager (the current SOP can be found in the approved SOP folder in the O drive).

To assure the safety of everyone, the trainee must read the Material Safety Data Sheet (MSDS) for information concerning each chemical used in the analysis. The toxicity levels and method of waste disposal should be clearly understood before beginning any analysis. Links to the MSDS(s) can be found on the TAMU Environmental Health and Safety Office web page. If you have difficulty locating a current MSDS contact the OTSC Safety Officer.

OBSERVE AND PRACTICE

Once familiar with the protocol and reagents, the trainee observes a qualified chemist perform the procedure.

To assure the trainee completely understands the analysis, (s)he must perform at least one practice set before beginning the actual qualifying sets. The practice set should consist of a variety of sample types and include challenging samples if available. Official qualification can begin if 1) working controls meet accuracy and precision requirements in the practice set and 2) the laboratory manager gives the trainee permission.

The trainee should consult with the laboratory manager to determine which specific reagents must be prepared for the analysis.

RUN QUALIFIYING SETS

The laboratory manager or his/her designee selects the samples to be run by the trainee. Samples may include regulatory samples, check samples, working control samples, standard reference materials, and blanks. It is preferable to use samples that have been run in duplicate and to use the average result for comparative purposes. The sets should consist of a variety of sample matrices. A minimum of three independent sets should be analyzed. Multiple sets may be run on the same day if each set has a unique instrument optimization and calibration curve.

QUALIFICATION INTERPRETATION

Sufficient Number of Samples Available-

Outliers will be evaluated and removed using the Dixon outlier test (see M0027). The remaining data will be compared using a paired t-test. If no differences are significant (P 0.05) then the chemist is qualified to run the analytical procedure. If there is a significant difference in the data (P < 0.05) then the Laboratory Manager should consult with the OTSC Quality Assurance Manager.

Insufficient Number of Samples Available-

Evaluation of data will depend on qualification procedure agreed upon by the laboratory managers.

Summary of Activities

- Review the procedures
- Observe its practices
- Practice two samples
- Qualification set of seven samples
- Final exam (unknown)