Aflatoxin Analysis:

Uncertainty

Statistical Process Control

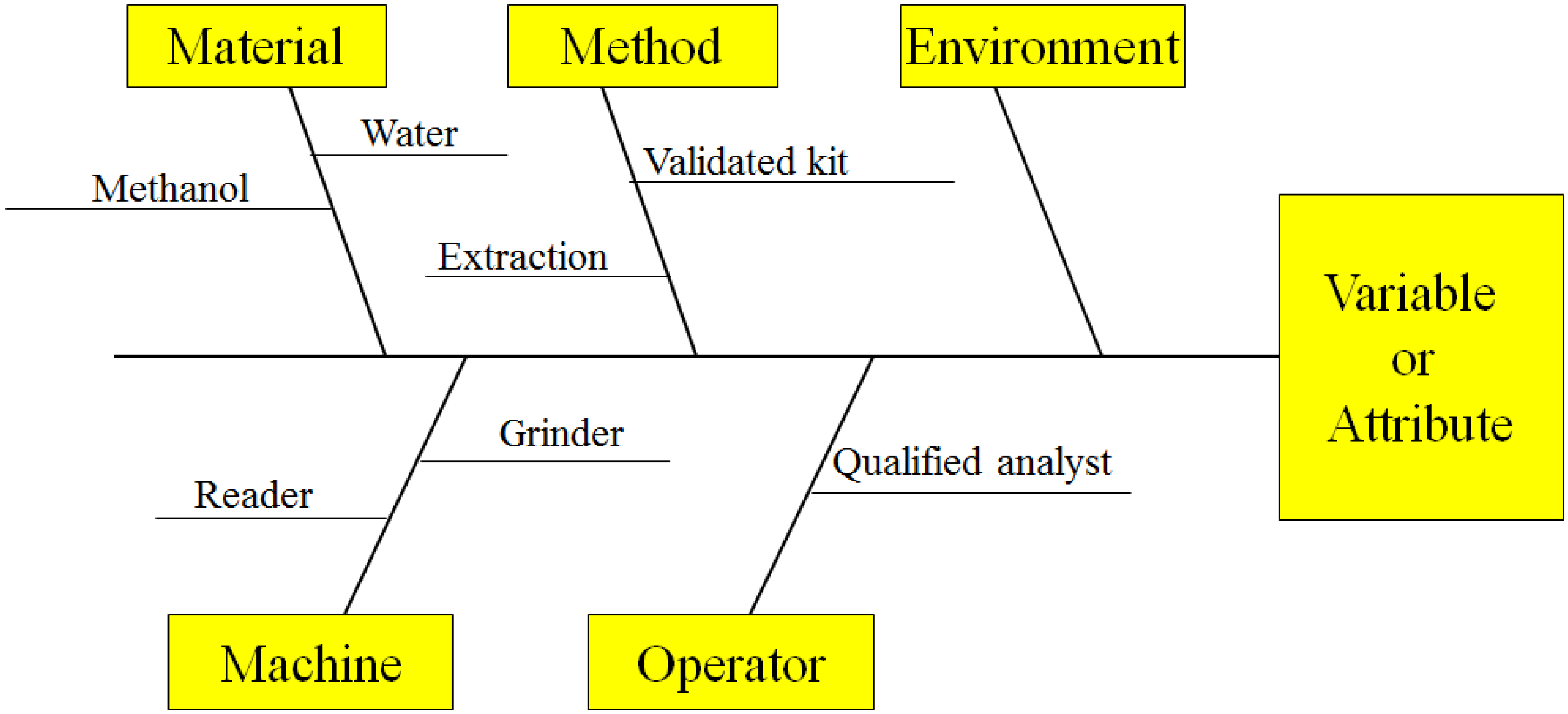
Sources of Variability

COMESA Session Five: Technical Courses November 18

Uncertainty

SOURCES OF VARIABILITY

Cause and Effect Diagram



Uncertainty Budget

The systematic description of uncertainty determinations relevant to specific measurements including ranges plus all factors, assumptions, and calculations included

Jim Balthrop

Basic Steps in Uncertainty Budgets

- List all potential factors affecting variability in measurements –make table
- Determine the standard uncertainty for each factor including distribution
- Perform root sum squares for all factors to create the combined or standard uncertainty
- Multiply by coverage factor

Jim Balthrop

Purpose

This procedure documents a procedure to calculate the uncertainty of reported analytical results in the laboratory.

Scope / Field of Application

This procedure applies to all analytical methods in the laboratory that run working control samples in duplicate with each set of samples.

Procedure

1. The mean, standard deviation, and coefficient of variation (CV) of the working control data is calculated. If 20 or more data points are available the uncertainty of measurement is determined by multiplying the CV by 2. (If there are less than 20 data points the CV is multiplied by 2.228). These coverage factors are for a 95% confidence.
2. Sources of variability will be evaluated and CVs calculated (e.g. weighing, pipetting, instrumentation, sample, testing kit column, and reagent.
3. *A computation of individual sources of variability will be compared with the total variation described in line 1.*

Reference

"P103b-Annex: Policy on Estimating Measurement Uncertainty for Life Sciences Testing Labs" The American Association for Laboratory Accreditation Sept 22, 2010, (see page 3)

Are you data rich and information poor?

STATISTICAL PROCESS CONTROL

Continuous Improvement Defined

A philosophy that strives for gradual, unending process improvement, through constant evaluation and employee involvement, by finding ways to do things better, and by setting and achieving increasingly higher standards.

Statistical Process Control

The application of statistical principles and techniques to all stages of production directed toward the most economical manufacture of a product.

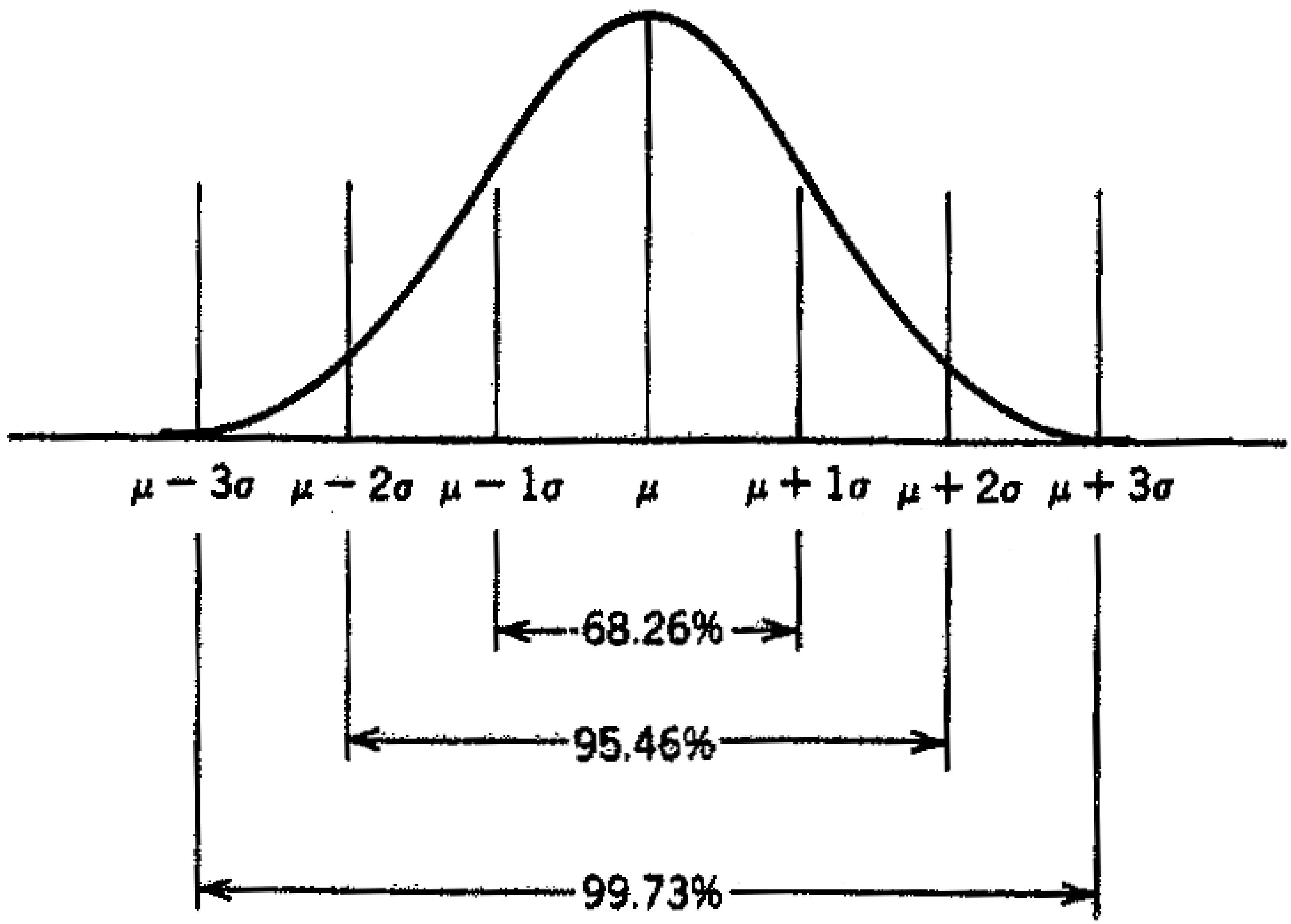
The economic benefits include increased product uniformity, less material rework and waste, improved production and plant efficiency, increased customer satisfaction resulting in repeat business, less money spent on finished product inspection, and fewer product recalls.

Goal of SPC

Elimination of variability in the process.

Variation and the Application of Statistics

- No two things are exactly alike.
- Variation in a product or process can be measured.
- Things vary according to a definite pattern.
- Whenever things of the same kind are measured, a large group of the measurements will tend to cluster around the middle.
- It's possible to determine the shape of the distribution curve.



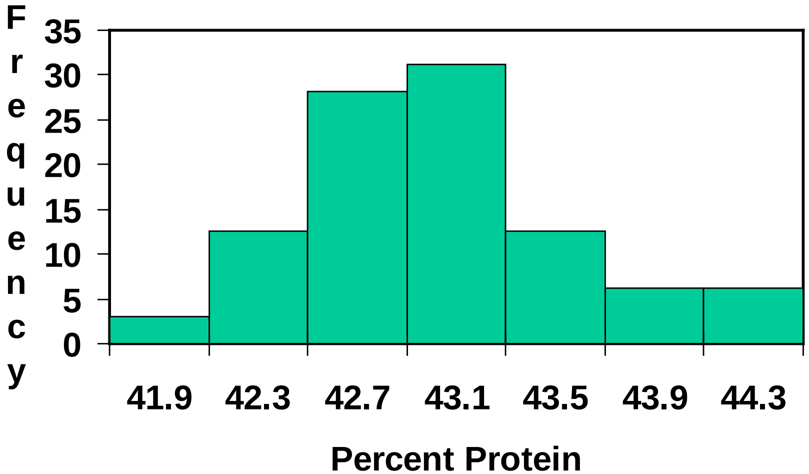
μ − 3σ
μ − 2σ
μ − 1σ
μ
μ + 1σ
μ + 2σ
μ + 3σ
68.26%
95.46%
99.73%

Type	Formula	Calculation
Mean	$\bar{x} = \frac{x_1 + x_2 + x_3 + \ldots + x_n}{n} = \frac{\sum x_i}{n}$ where: $\bar{x}$ = mean $x_1 + x_2 + x_3 \ldots$ = individually measured value (x_1) n = number of measurements	$\frac{1+2+3}{3} = 2$ $x_1 = 1,\ \ x_2 = 3;\ \ x_3 = 3$
Variance	$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{h} x_i\right)^2}{n}\right]$	$1 = 1/2[14 - \frac{6^2}{3}]$
Standard Deviation	$s = \sqrt{s^2}$	$1 = \sqrt{1}$
Coefficient of Variation	$\%\,CV = \frac{s}{\bar{x}} \text{ x } 100$	$50\% = 1/2 \times 100$

STATISTICAL TOOLS

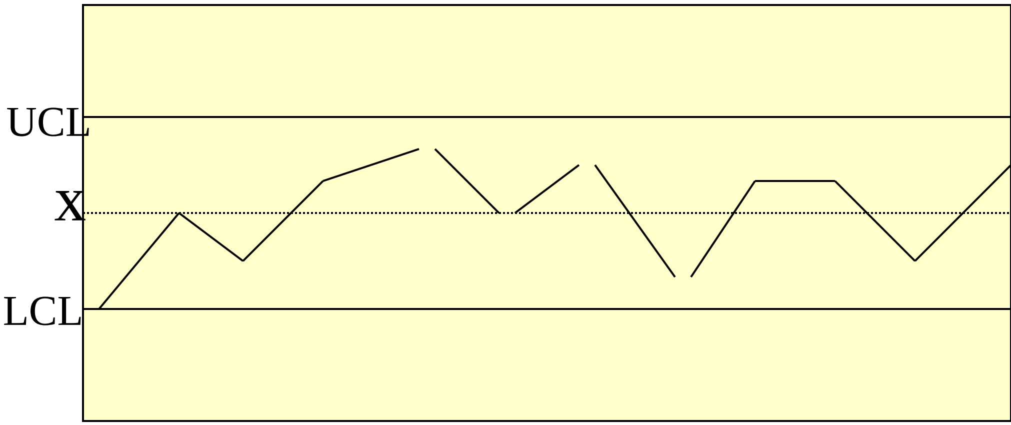
<u>Histogram</u>

Process/Product Distribution
Process Centered
Process Capability
Economic Performance



<u>Control Chart</u>

Differentiates type of variability
Improves Profitability
Effective for Defect Prevention
Avoid Unnecessary Adjustment



Control Charts

A control chart contains a record of the results of periodic inspections over time. The control chart is popular in many industries for the following reasons.

1. Control charts are proven techniques for improving productivity
2. Control charts are effective in defect prevention.
3. Control charts help prevent unnecessary process adjustments.
4. Control charts provide diagnostic information.
5. Control charts provide information about process capability.

Steps in preparing a control chart with repeated measures

1. Collect samples (in this e.g. 5 bags from 20 lots).
2. Calculate mean and range for sub-samples,
3. Calculate sum and average for mean and range data.
4. Calculate the control limits for the mean and range

Mean

First, select the A_2 value based on the number of sub-samples
Second, multiply the average range by the A_2 value
Third, add the product to the average Mean for the UCL
Fourth, subtract the product from the average Mean for the LCL

Range

First, select the D_4 value based on the number of sub samples.
Second, multiple the average range by the D_4 value

Factors for Control Limits (ASQC)

N	A_2	D_4	d_2	$3/d_2$
2	1.880	3.268	1.128	2.659
3	1.023	2.574	1.693	1.772
4	0.729	2.282	2.059	1.457
5	0.577	2.114	2.326	1.290
6	0.483	2.004	2.534	1.184

						Mean	Range
1	40.00	40.20	40.05	40.00	40.10	40.07	0.20
2	40.10	40.17	40.15	40.20	40.00	40.12	0.20
3	39.90	39.95	39.95	40.05	40.00	39.97	0.15
4	40.05	40.10	40.10	40.05	40.03	40.07	0.07
5	40.00	40.10	40.10	40.05	40.10	40.07	0.10
6	40.25	40.15	40.25	40.15	40.15	40.19	0.10
7	40.30	40.10	40.10	40.30	40.30	40.22	0.20
8	40.05	40.10	40.05	40.05	40.25	40.10	0.20
9	40.10	40.10	40.10	40.20	40.20	40.14	0.10
10	40.10	40.10	40.05	40.20	40.05	40.10	0.15
11	40.30	40.20	40.15	40.05	40.05	40.15	0.25
12	40.15	40.30	40.15	40.20	40.20	40.20	0.15
13	40.00	40.05	40.05	40.05	40.00	40.03	0.05
14	40.10	40.10	40.04	40.25	40.25	40.15	0.15
15	40.00	40.10	40.10	40.10	40.00	40.06	0.10
16	40.12	40.10	40.10	40.40	40.00	40.14	0.40
17	40.12	40.27	40.25	40.10	40.15	40.18	0.17
18	40.10	40.10	40.00	40.20	40.10	40.10	0.20
19	40.30	40.20	40.15	40.15	40.20	40.20	0.15
20	40.15	40.30	40.10	40.20	40.15	40.16	0.20
					Total	802.42	3.29
					Avg.	40.12	0.1645

Factor for averaged values in a variable control chart

$$A_2 = \frac{3}{d_2\, n^{.5}}$$

$$UCL = \bar{\bar{x}} + A_2\bar{R}$$

$$\text{Center line} = \bar{\bar{x}}$$

$$LCL = \bar{\bar{x}} - A_2\bar{R}$$

Where:
3 represents the number of standard deviations above and below mean

n represents number of Repeated measures.

Factors for Control Limits (ASQC)

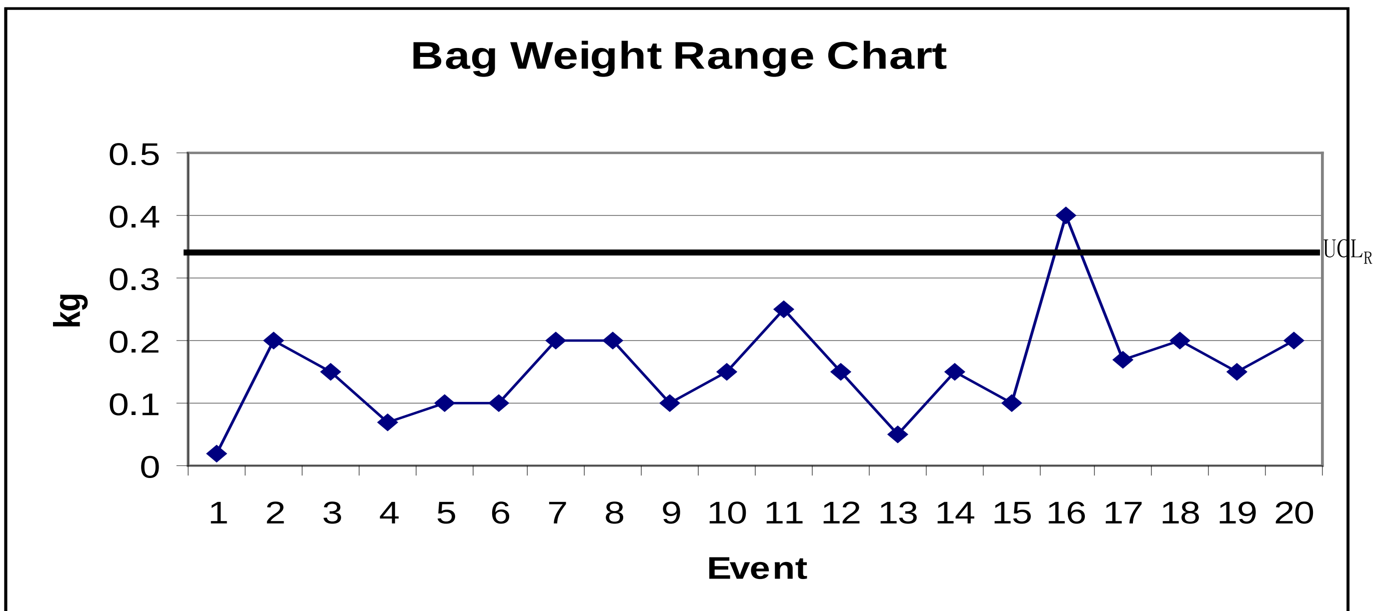
N	A_2	D_4	d_2	$3/d_2$
2	1.880	3.268	1.128	2.659
3	1.023	2.574	1.693	1.772
4	0.729	2.282	2.059	1.457
5	0.577	2.114	2.326	1.290
6	0.483	2.004	2.534	1.184

Variable Control Chart Range Statistics

$UCL_R = D_4 \times \bar{R}$

$= 2.114 \times 0.1645$

$UCL_R = 0.348$



Variable Control Chart Average Statistics

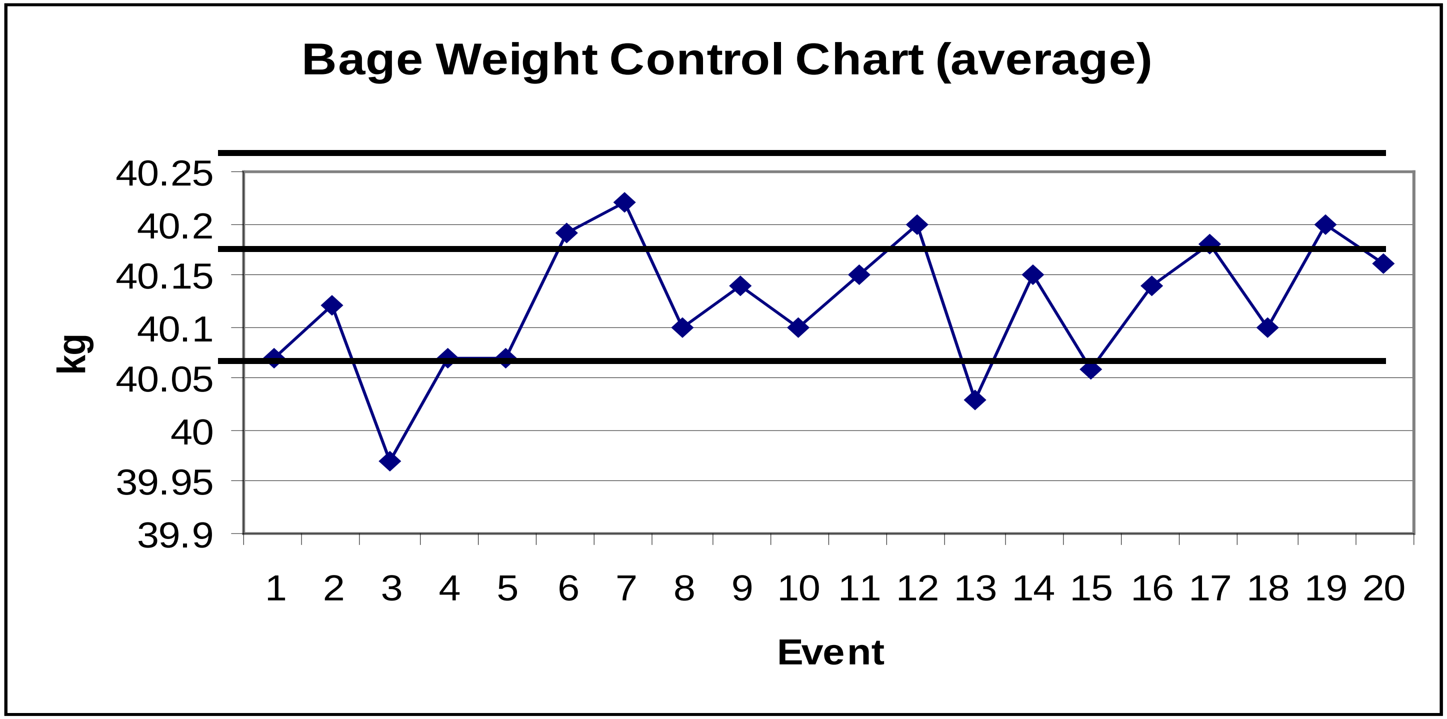
Average Mean = 40.121

Average $\bar{R}$ange = 0.1645

A_2 time R = 0.577 x 0.1645 = 0.0949

$UCL_{\bar{x}}$ = 40.121 + 0.0949 = 40.21

$LCL_{\bar{x}}$ = 40.121 – 0.0949 = 40.03



Interpreting a Control Chart

The process is considered out of control if any one or more of the criteria is met:

- One or more points outside of the control limit
- A run of at least eight points, where the type of run could be either a run up or down, a run above or below the center line, or a run above or below the median.
- Two of three consecutive points outside the 2-sigma warning limits but still inside the control limits.

Interpreting a Control Chart cont.

- Four of five consecutive points beyond the 1-sigma limits.
- An unusual or non-random pattern of the data.
- One or more points near a warning or control limit.

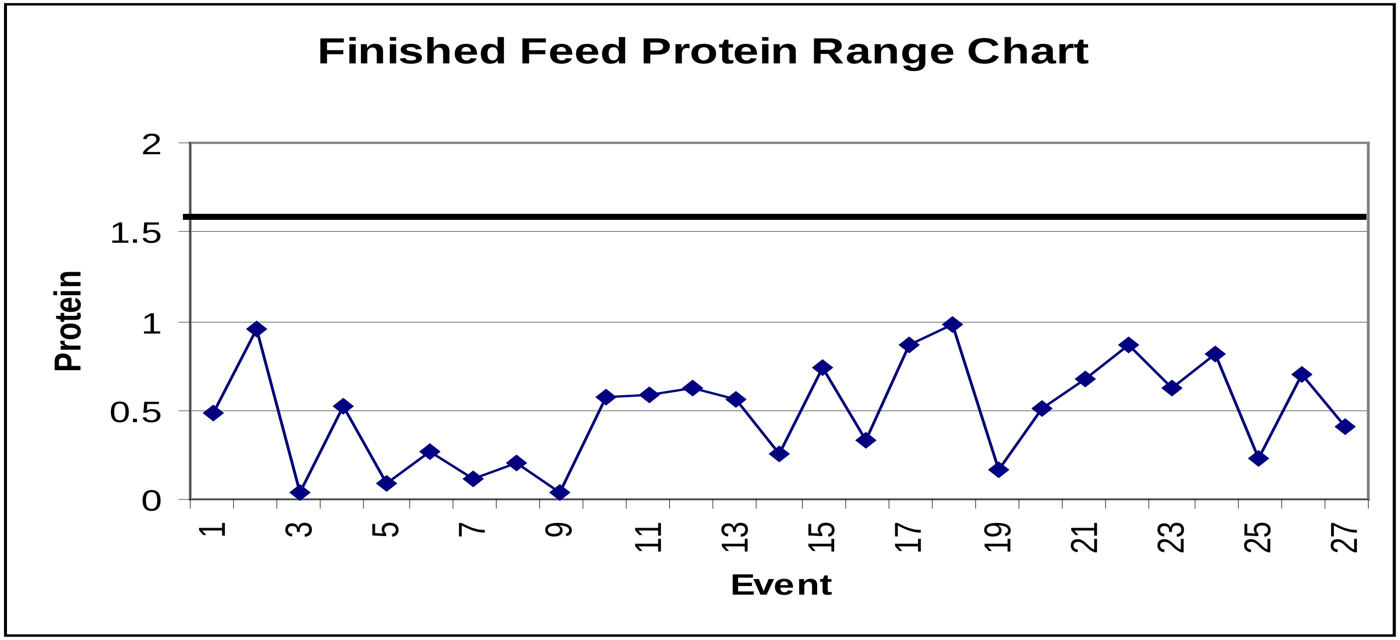
Batch Number	Protein Content	Moving Range
1	17.47	
2	17.95	.48
3	18.91	.96
4	18.87	.04
5	18.35	.52
6	18.44	.09
7	18.71	.27
8	18.60	.11
9	18.80	.20
10	18.84	.04
11	19.41	.57
12	18.82	.59
13	18.19	.63
14	18.75	.56
15	19.01	.26
16	18.27	.74
17	18.60	.33
18	19.46	.86
19	18.48	.98
20	18.24	.16
21	17.73	.51
22	18.40	.67
23	19.26	.86
24	18.64	.62
25	19.46	.82
26	19.23	.23
27	18.53	.70
28	18.12	.41
Total	521.54	13.21
Average	18.62	0.489

Variable Control Chart Range Statistics

$$UCL_{\bar{R}} = D_4 \times \bar{R}$$

$$= 3.268 \times 0.489$$

$$UCL_{\bar{R}} = 1.6$$



Mean = 18.62

Moving Range = 0.489

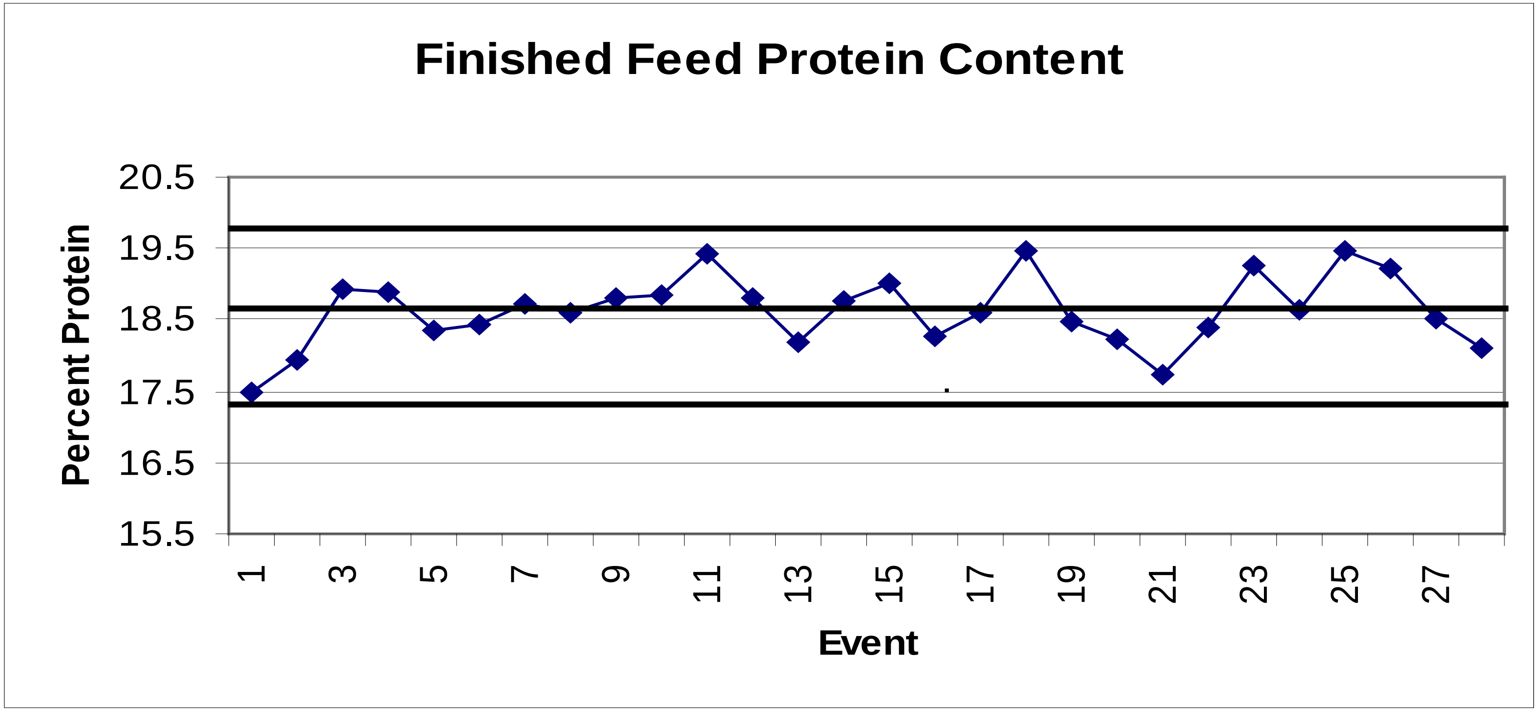
$UCL_x = x + 3(MR/d_2)$

Center line = x

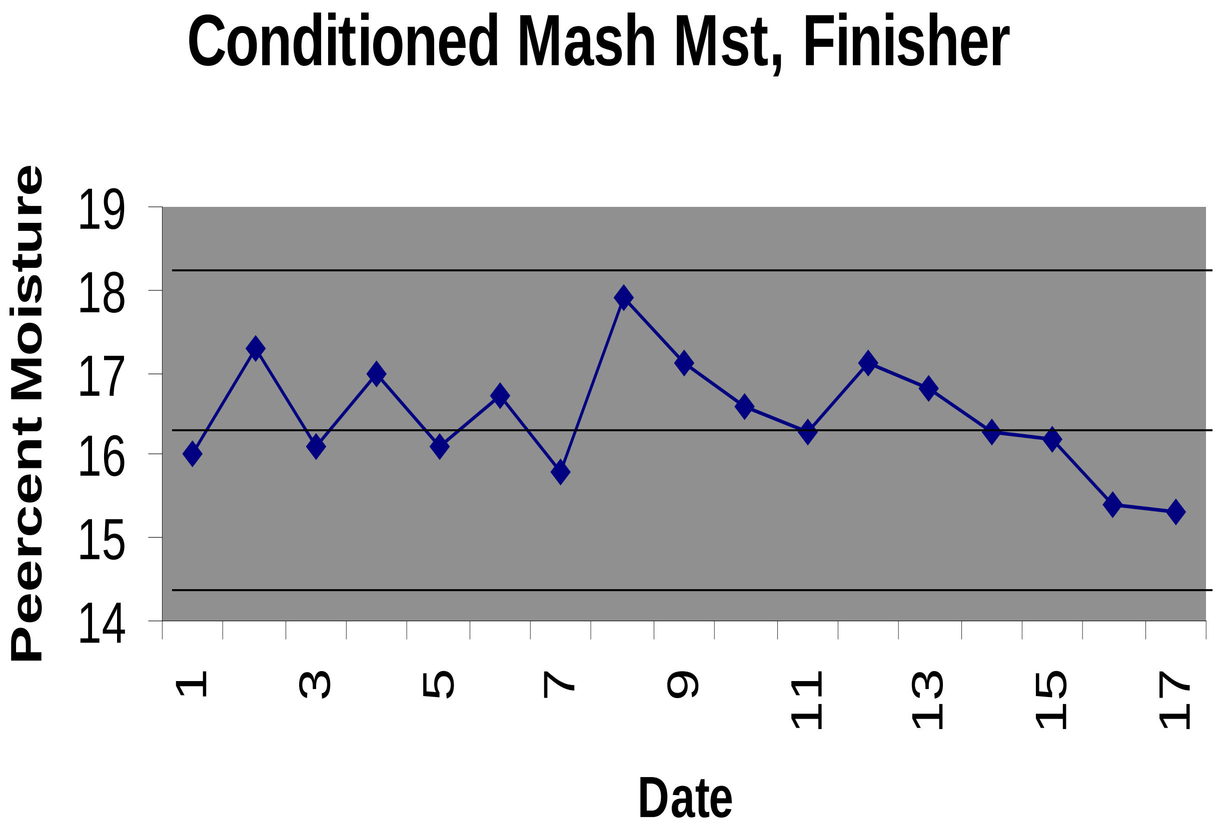
$LCL_x = x - 3(MR/d_2)$

$UCL_x = 18.62 + 3(.489/1.128) = 19.92$

$LCL_x = 18.62 - 3(.489/1.128) = 17.32$



Conditioner	
Moisture	
16.2	
16	
17.3	1.3
16.1	1.2
17	0.9
16.1	0.9
16.7	0.6
15.8	0.9
17.9	2.1
17.1	0.8
16.6	0.5
16.3	0.3
17.1	0.8
16.8	0.3
16.3	0.5
16.2	0.1
15.4	0.8
15.3	0.1
280	12.1
16.5	0.76
UCL	18.48
LCL	14.46



Designing a Control Chart and Factors to Consider

- Control Limit
- Sample Size
- Frequency (how often)

SPC & Testing a Hypothesis

Testing the hypothesis that the process is under statistical control

Point plotting with the control limits is equivalent to failing to reject the null hypothesis (in statistical control).

Plotting points outside the control limits is reject Ho.

Type I - process out of control when it is in control

Type II - concluding the process is in control when it is really out of control.

Moving the control limits further from the center line will decrease Type I error.