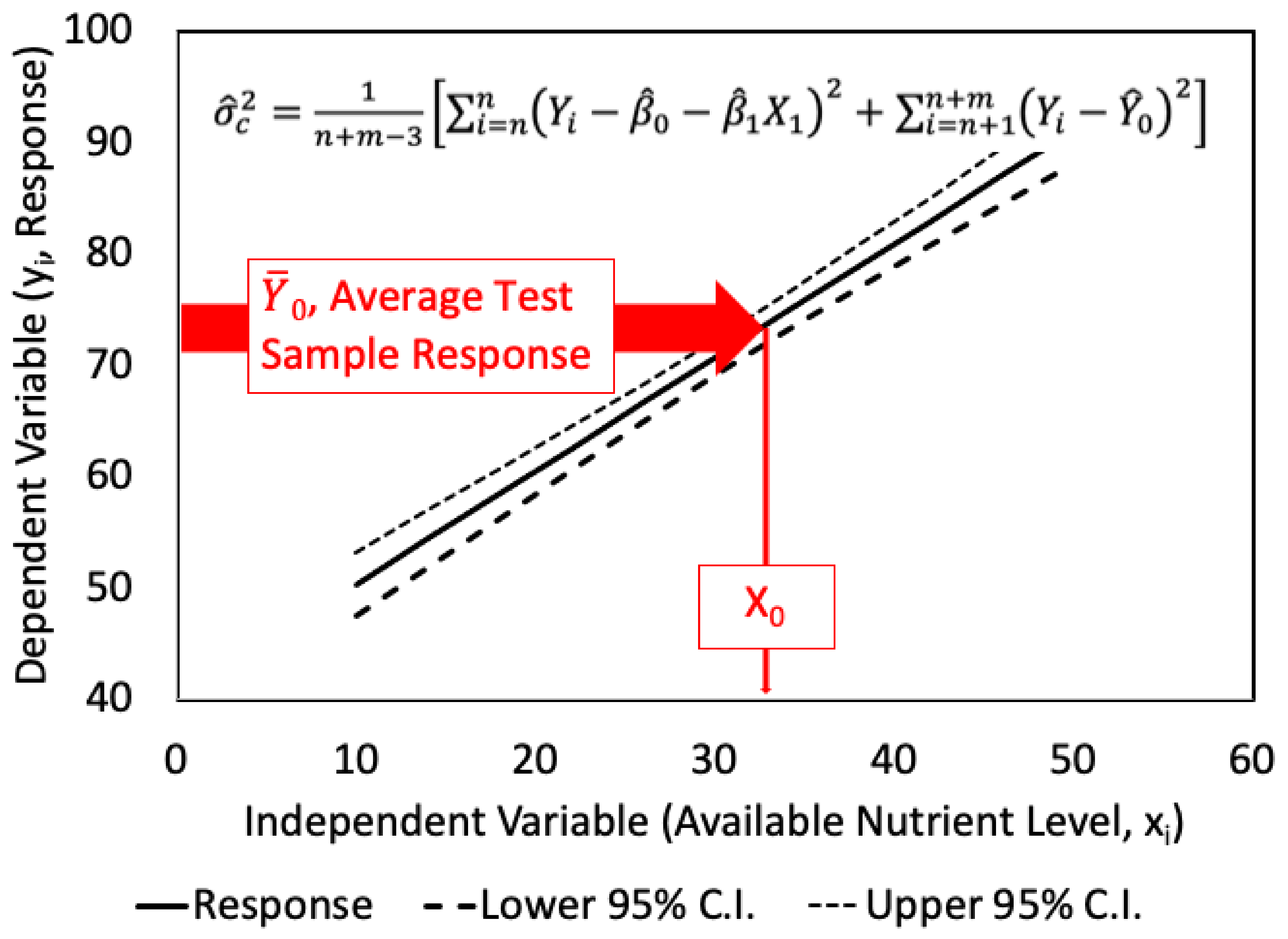
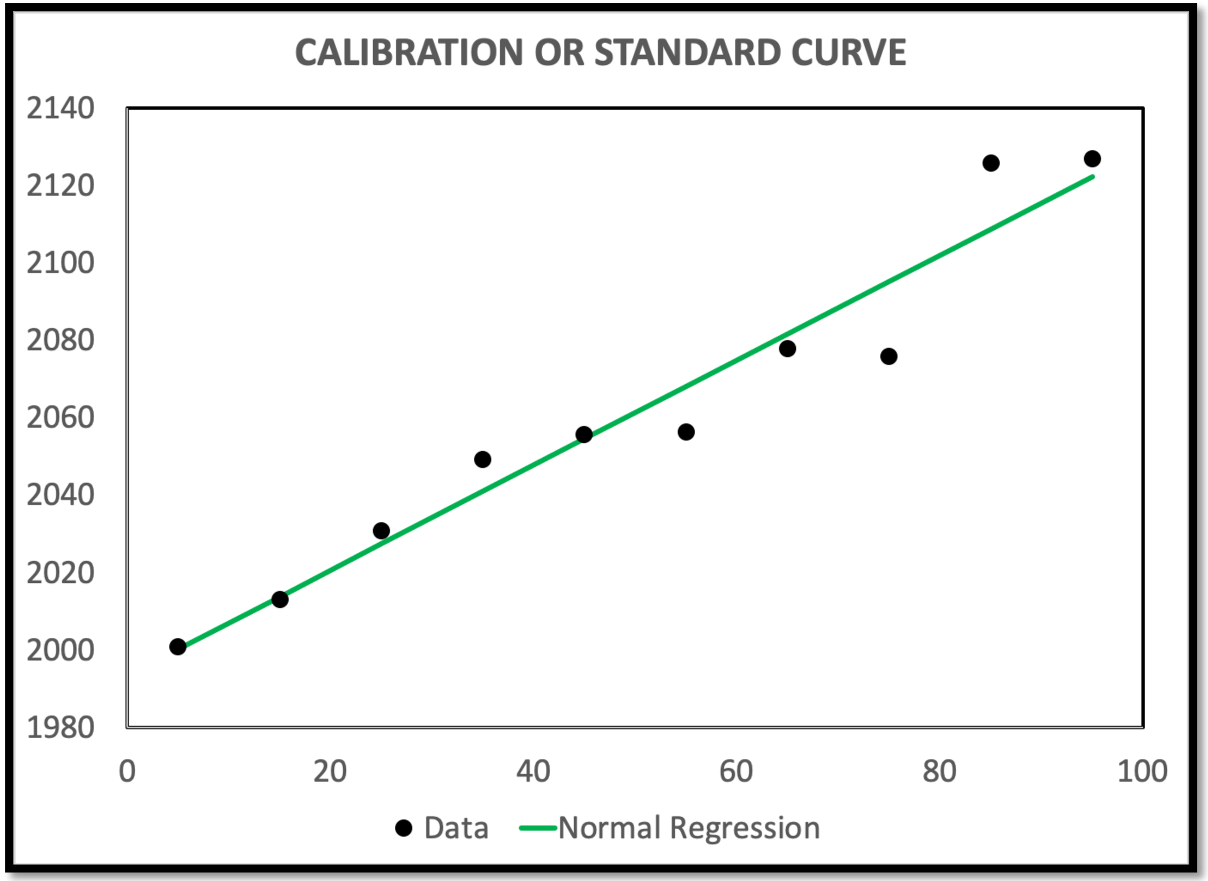
Abductive Statistical Methods Improve the Results of Calibration Curve Bioassays



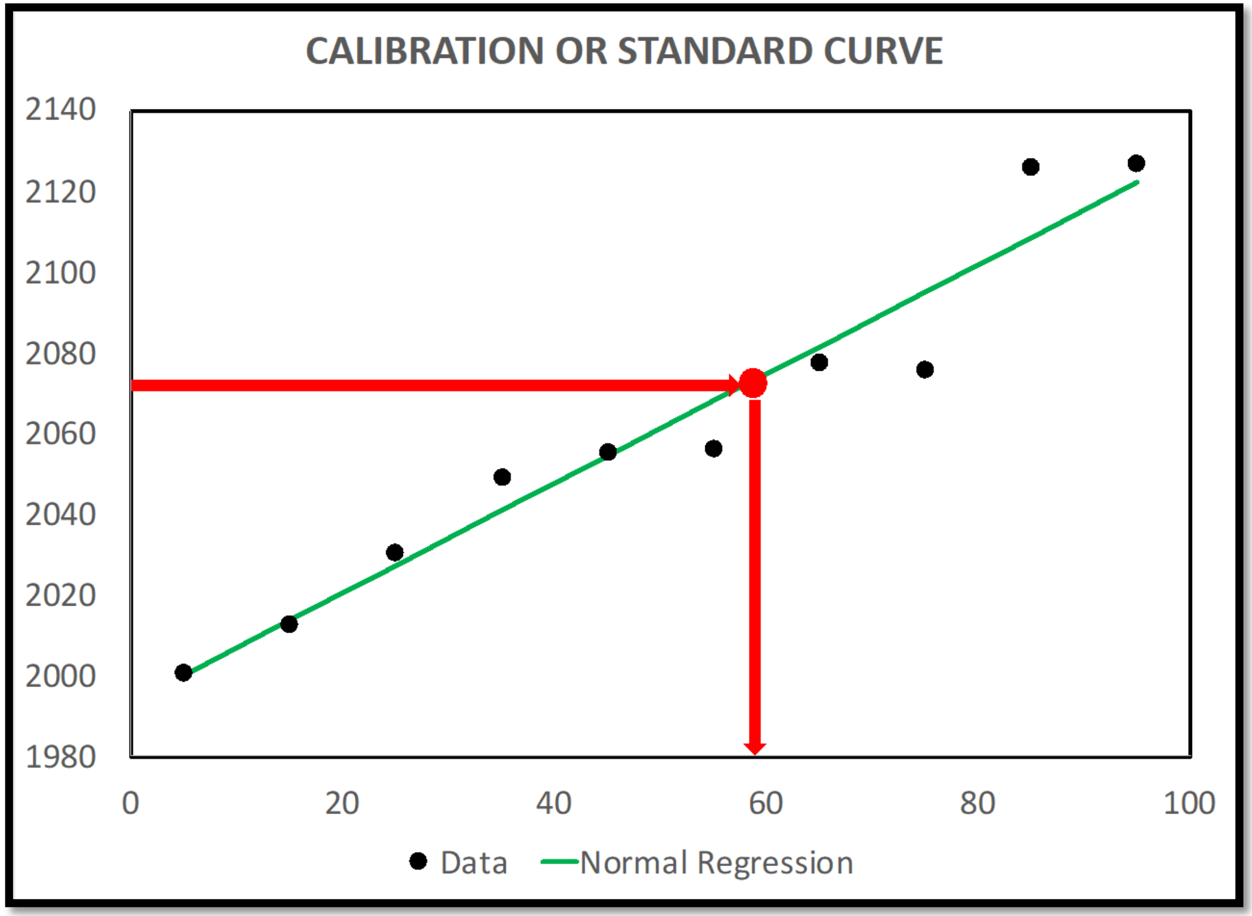
The Calibration Curve Problem

- Also called the standard curve problem
- Method to quantitate composition of unknown or test samples
- Compare standards to test samples
- Regress results from standards



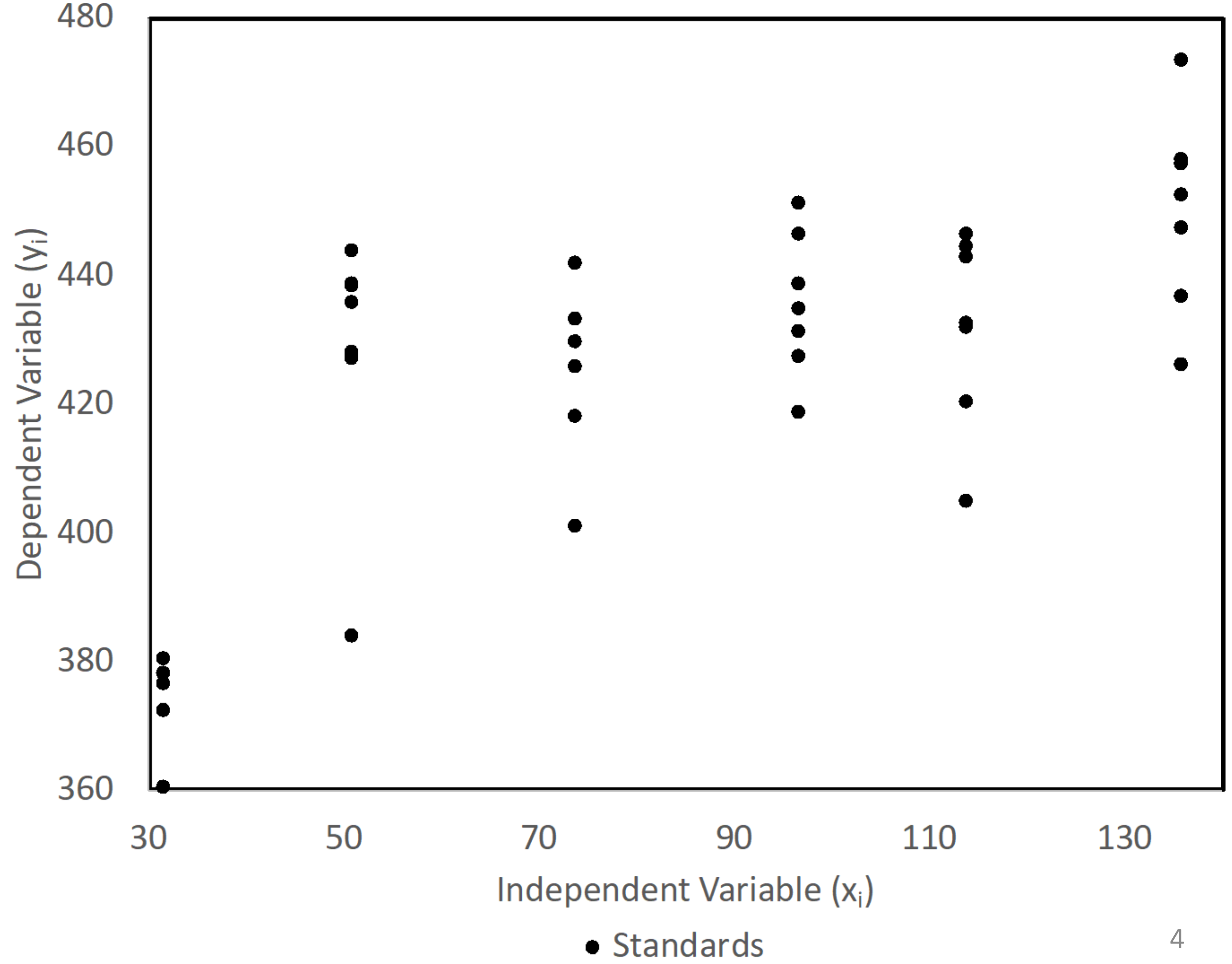
The Calibration Curve Problem

- Also called the standard curve problem
- Method to quantitate composition of unknown or test samples
- Compare standards to test samples
- Regress results from standards
- Predict “x” from results of test samples “y”



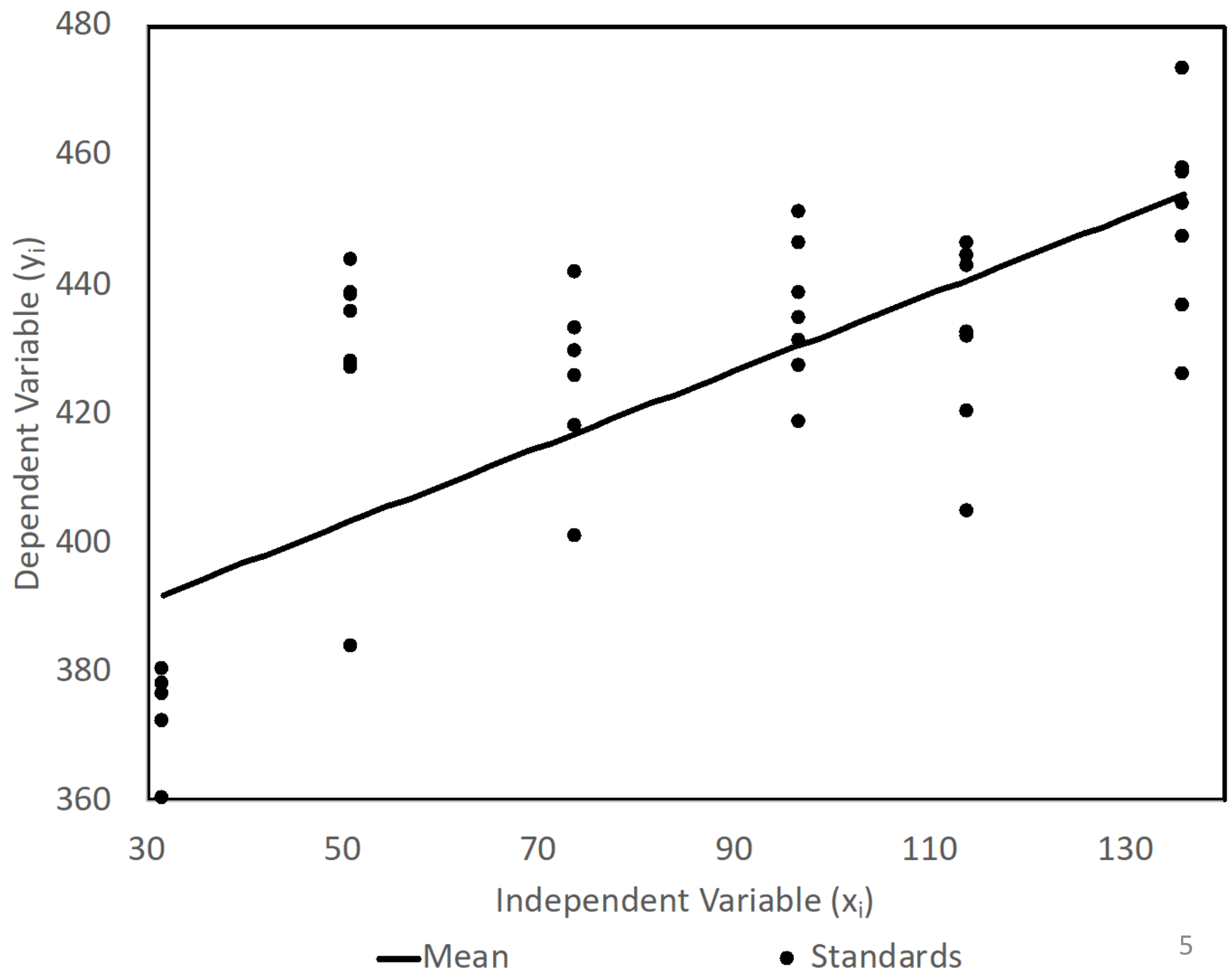
Calibration Curves: General Considerations

- Begin by finding the responses of the dependent variable to the independent variable
- Dependent variable
  - Standards from some known source
  - Measured properties or values



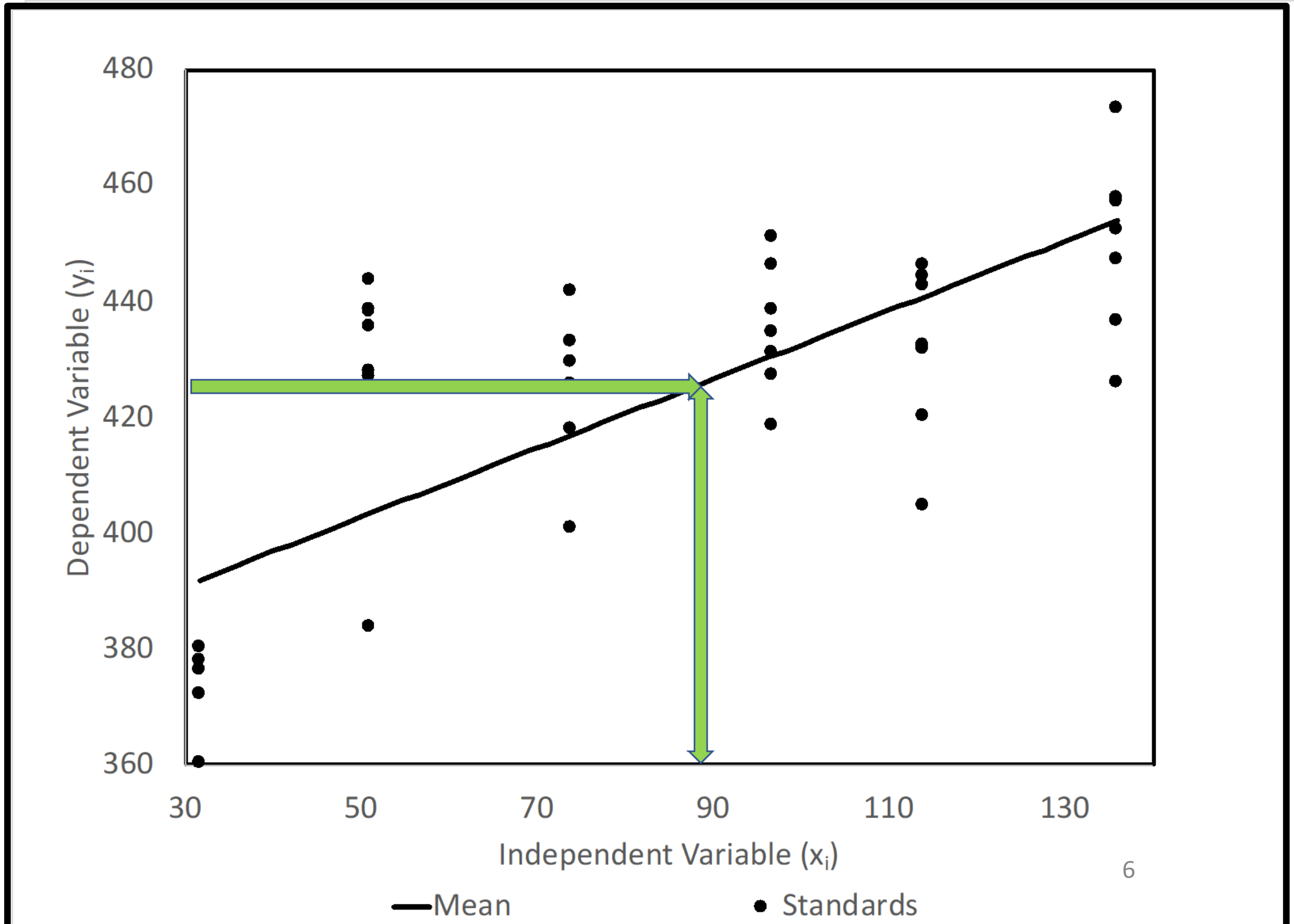
Calibration Curves: General Considerations

- Then fit a calibration, or standard curve
- By convention:
  - $Y_i = b_1 x_i + b_0$



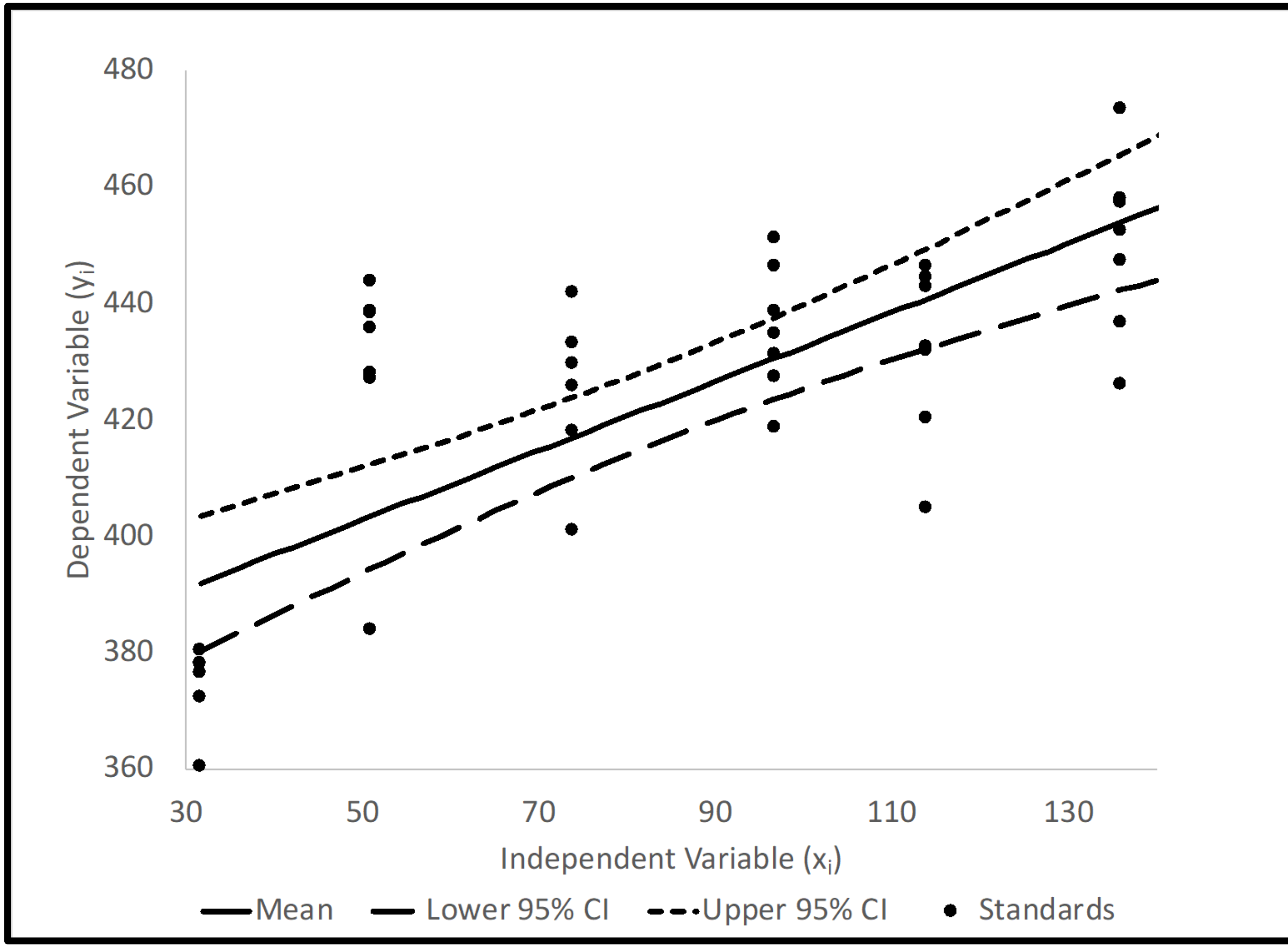
Calibration Curves: General Considerations

- Next measure the response of a test sample of unknown composition
- Calculate it's independent variable (x_0) composition
  - $x_0 = (y_0 – b_0)/b_1$



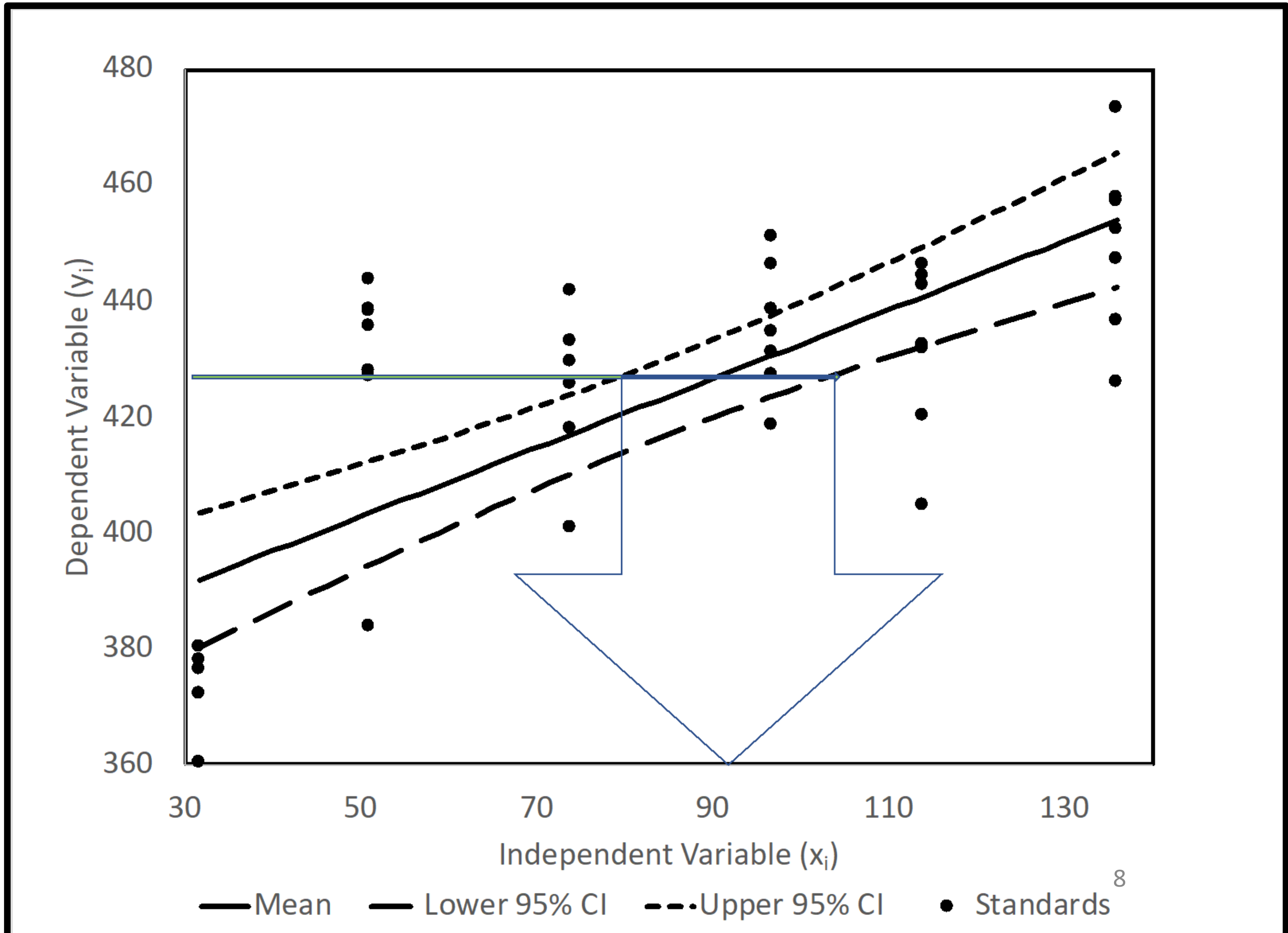
Calibration Curves: General Considerations

- Calibration curves are not measured without error
- Confidence intervals may be calculated
  - Ci's are minimal at the mid-point of the independent variable values



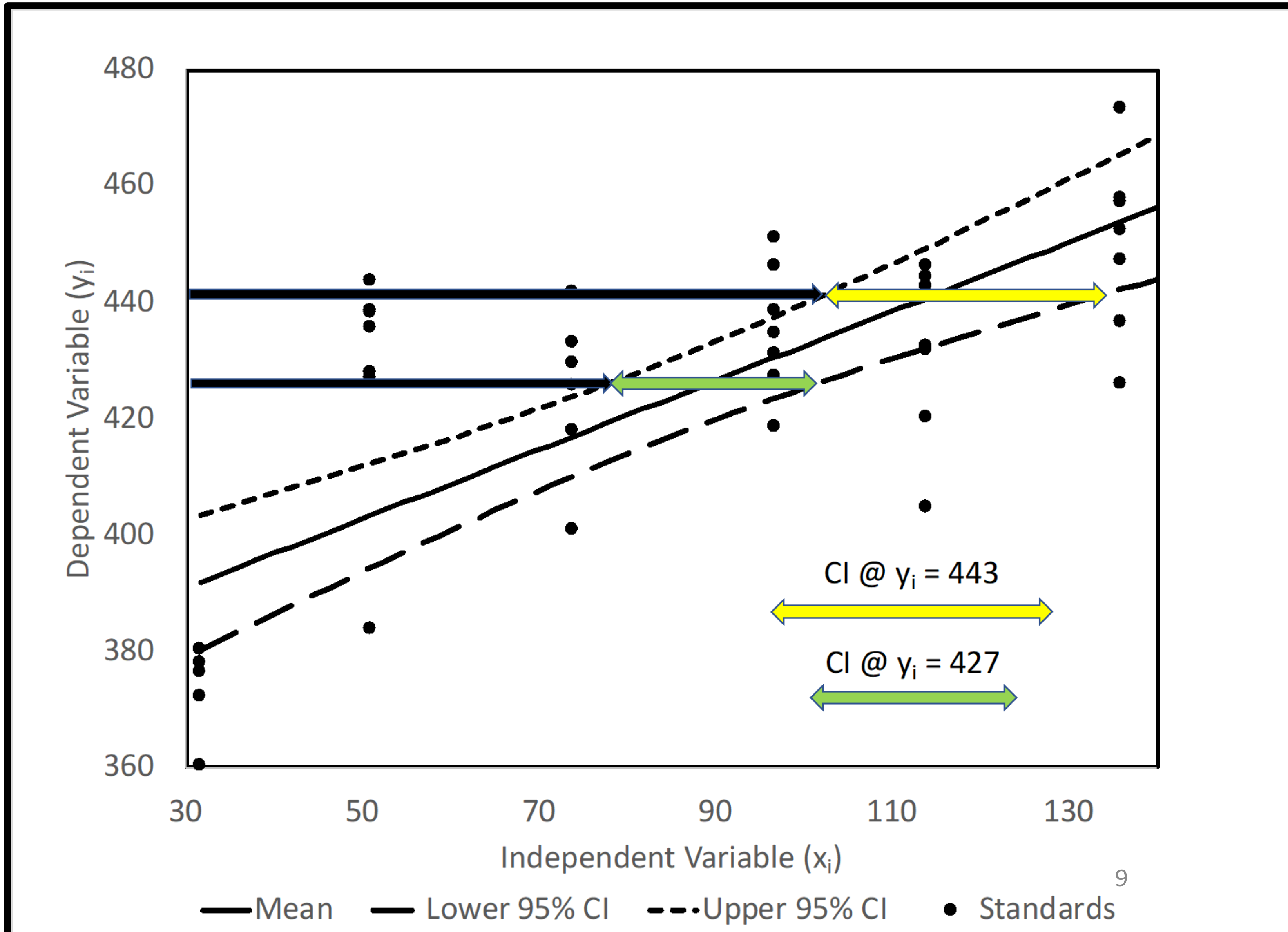
Calibration Curves: General Considerations

- Because of error in the standard curve
  - The real estimate of x_0 can only be described as being within some confidence interval
  - The estimated mean
    - Has a 50% chance of being above the mean value
    - Has a 50% chance of being below the mean value



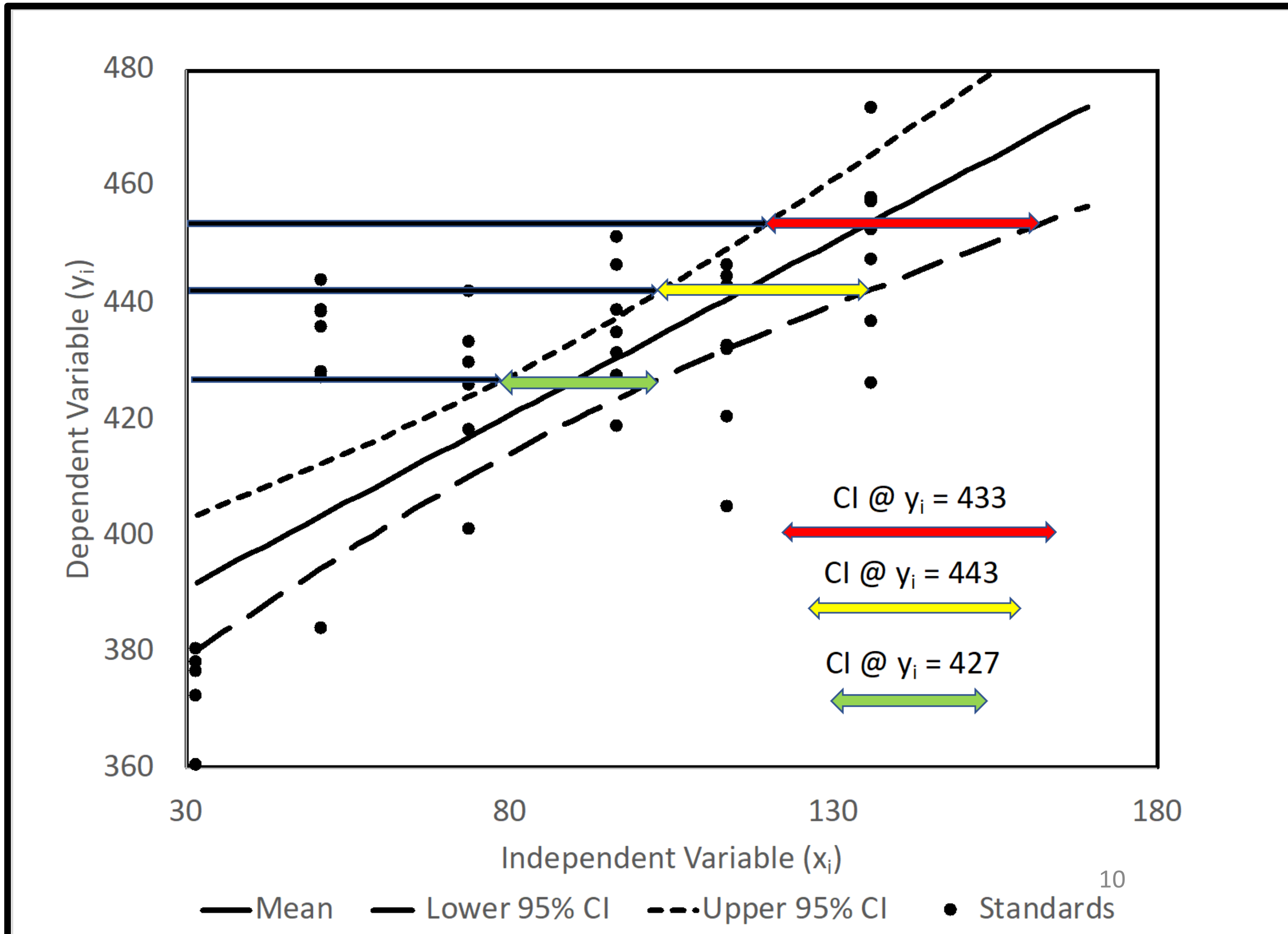
Calibration Curves: General Considerations

- The confidence intervals of predicted x_0's should be different for test samples near the center and extremes of the calibration curve



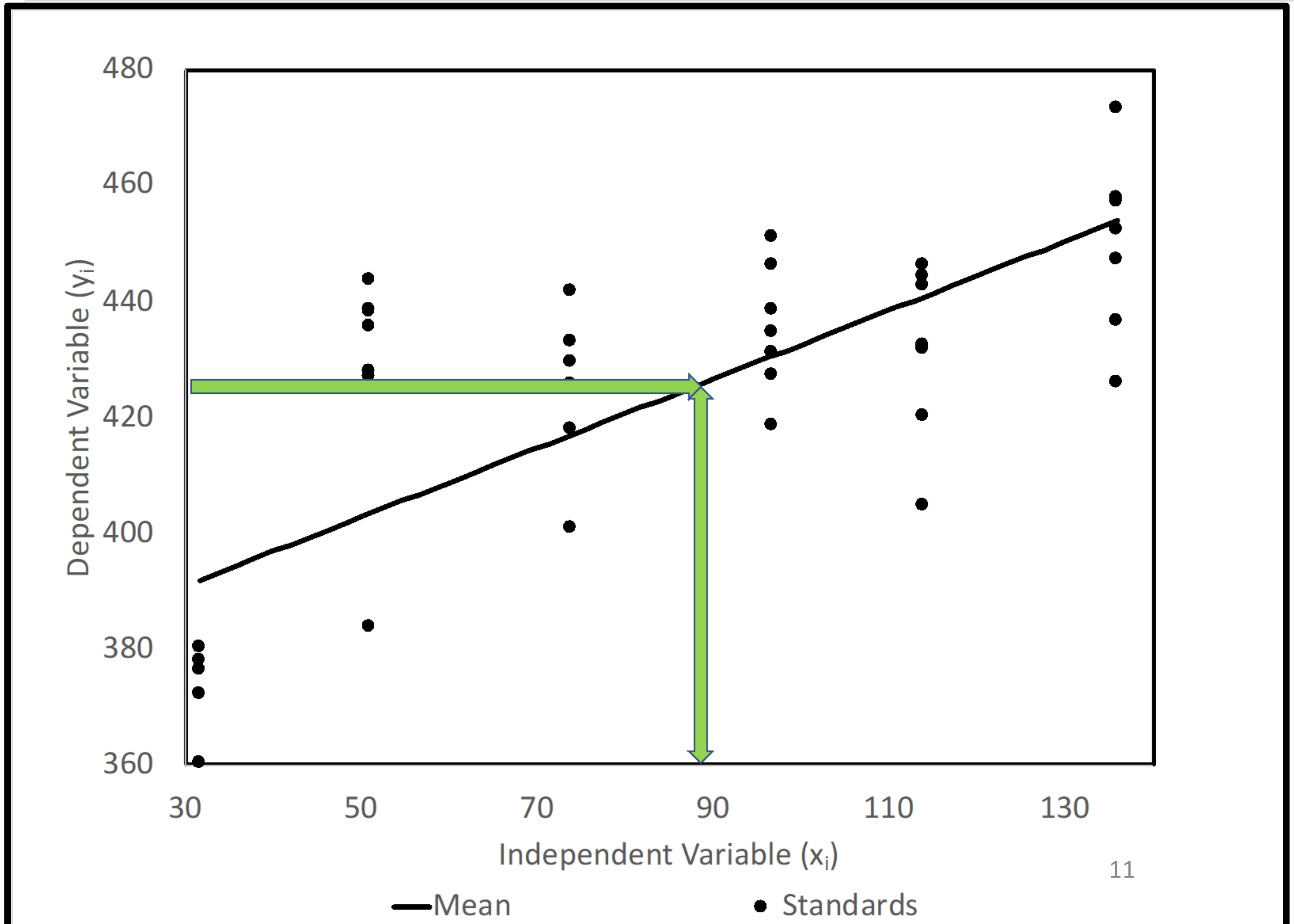
Calibration Curves: General Considerations

- The farther from the center of the calibration curve, the greater the ci's will be
- Recommendations are usually to keep the mean test sample values in the same range as the standards
- Extra care may be necessary when CI's go outside the range of the standard curve as well



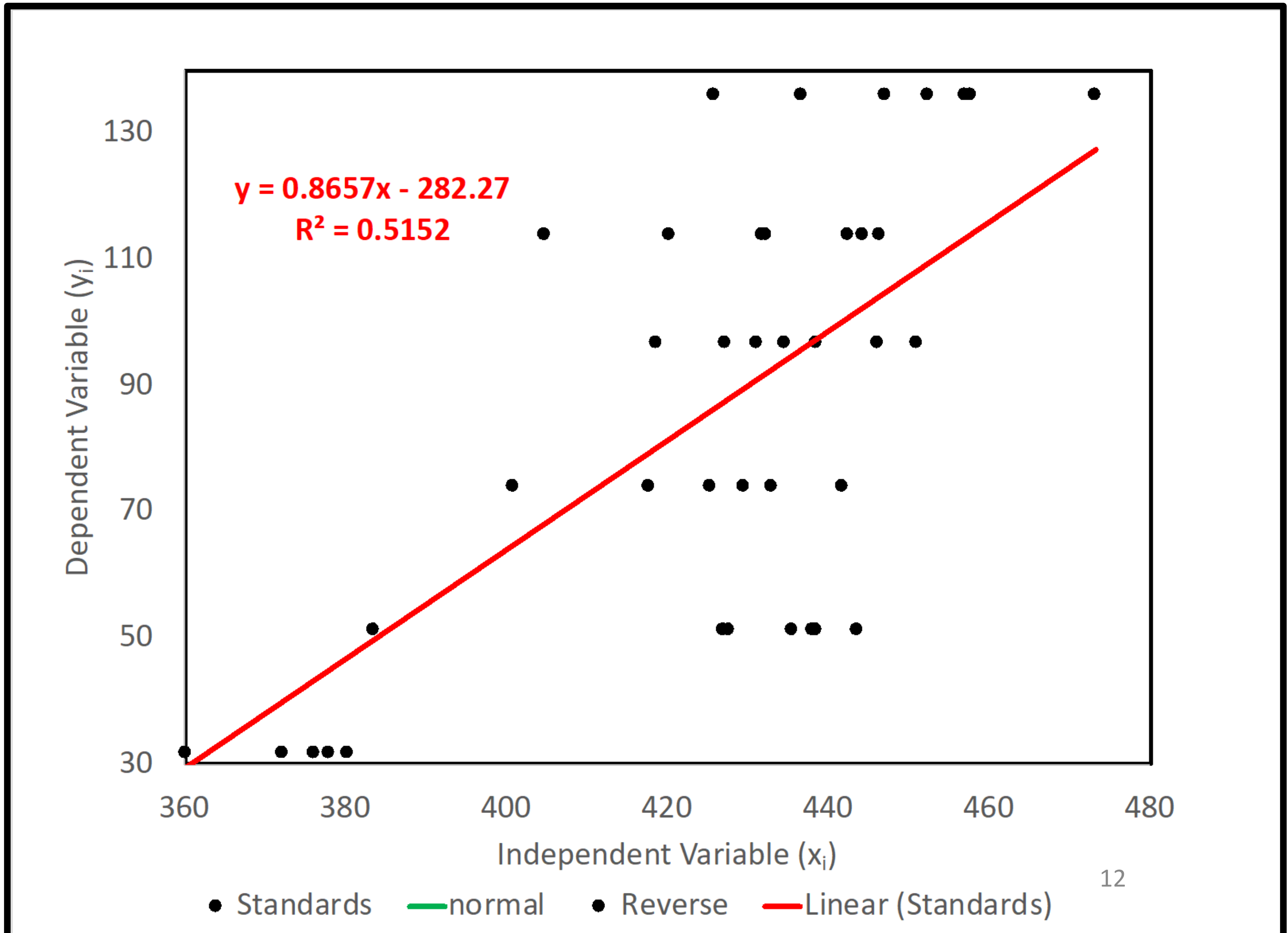
Calibration Curves: General Considerations

- Theoretical conundrum?
  - Belief that x is the independent variable
    - $y_i = b_1 x + b_0$
  - Then use inverted equation to solve for x
    - $x_0 = (y_0 - b_0)/b_1$
  - In reality finding x=f(y)



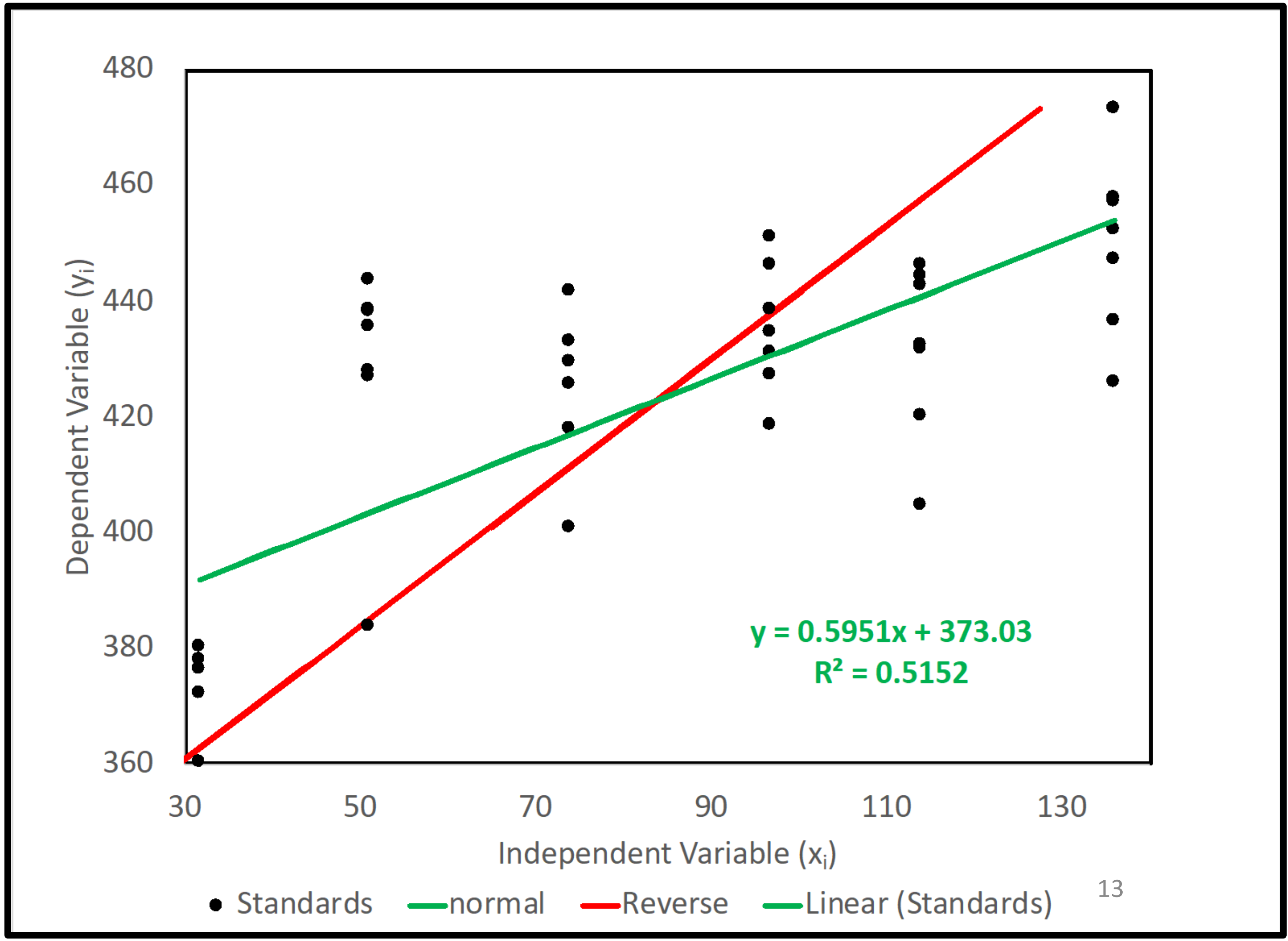
Calibration Curves: General Considerations

- If x were considered a function of y, the resulting standard curve would be different !
- But it would have the same R^2 !
- The reverse regression line goes through (360,30)
- Note that the variables and scales have been reversed, but not the labels
- The equation is as excel presented it



Calibration Curves: General Considerations

- The normal and reverse regression lines graphed in normal (x,y) space
- Note how the reverse regression line now goes through (30,360)
- While a broken clock gives the correct time twice per day, reverse regression only gives the right answer once in infinity



What ***Is*** The Problem?

- Typically
  - Researchers ignore error in the standard curve
  - Make x the independent variable with no associated error
    - Then try to estimate the error in x ???
  - Researchers predict one value of x for each replicate of y
    - If there are 3 replicates they make 3 predictions with zero (0) degrees of freedom each, etc.

What ***<u>Is</u>*** The Problem?

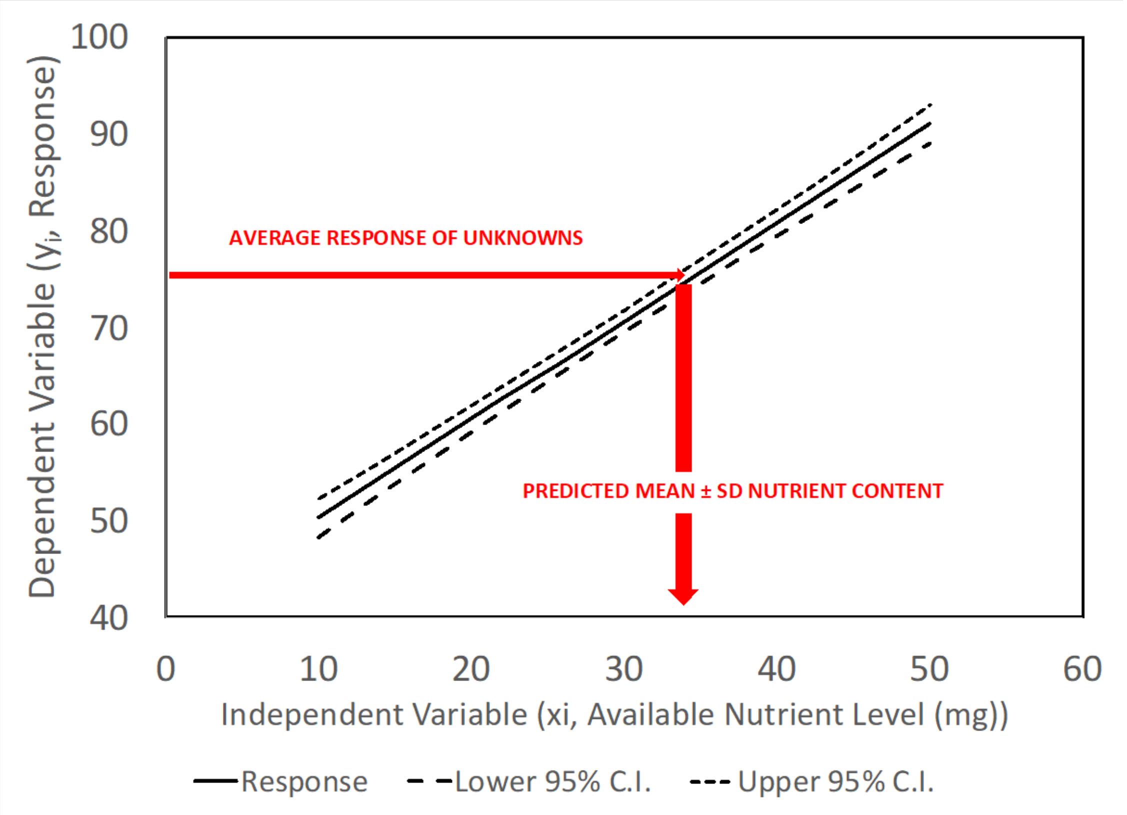
- Results with the classical standard curve method are:
  - Precise
  - Reliable
  - Repeatable
- The results make us
  - Happy
  - Satisfied

What ***Is*** The Problem?

What do happy & staisfied have to do with research ???

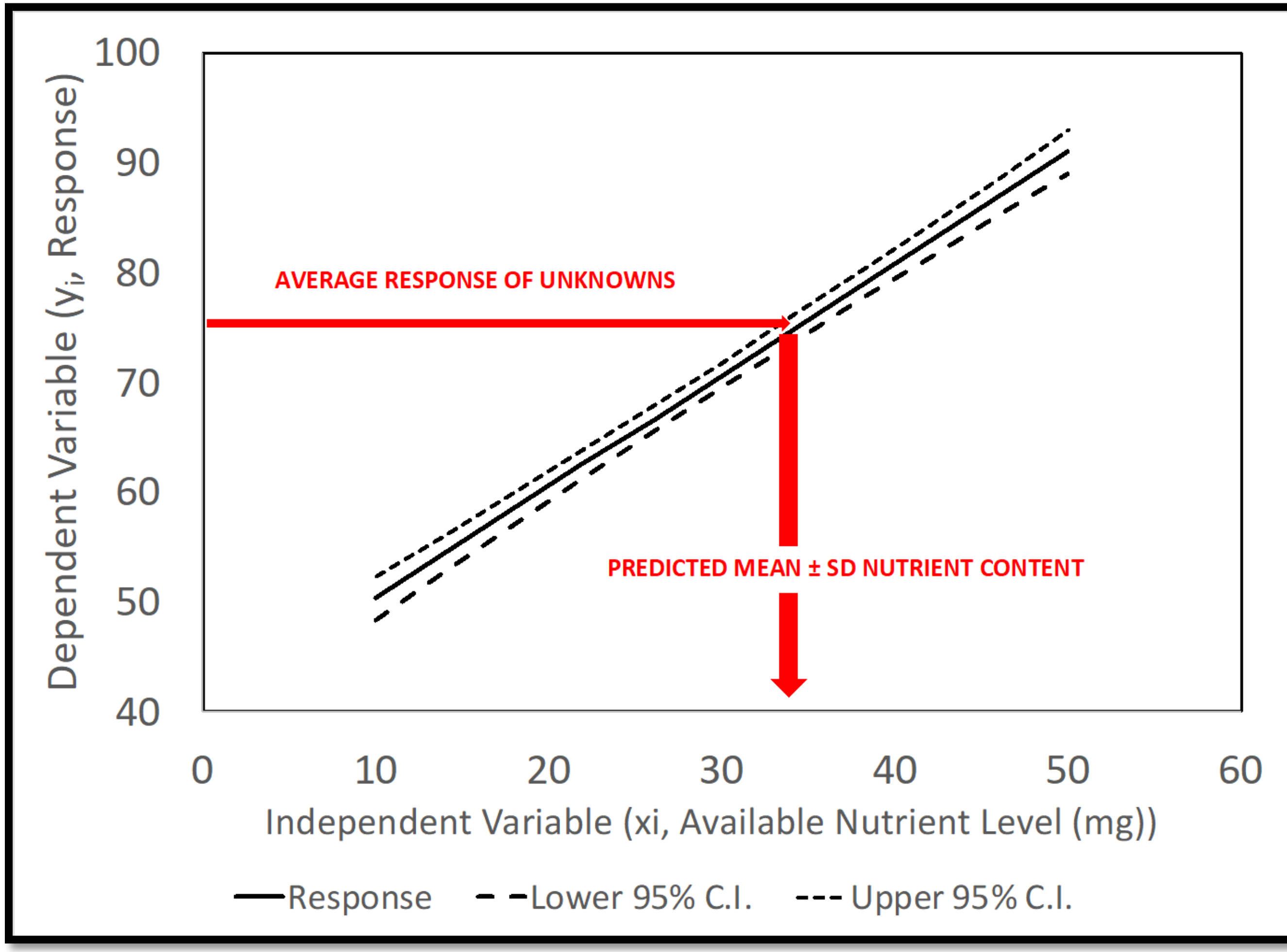
What ***Is*** The Answer?

- There are other methods of predicting the x value of test samples
- They are not perfect either
  - There is no direct, exact method of determining the confidence level of the composition of a test sample
- Alternate methods are based on observations and theories
- They give the same or better results than the classical, or intuitive method near the center of the calibration curve
  - With 3 replicates, one mean estimate with 2 degrees of freedom



Is Changing Methods Worth The Effort?

- **Effort, what effort?**
- Once the calculations are programmed, there is no effort
- **Only a chance to present data properly with smaller confidence intervals when appropriate !!!!**

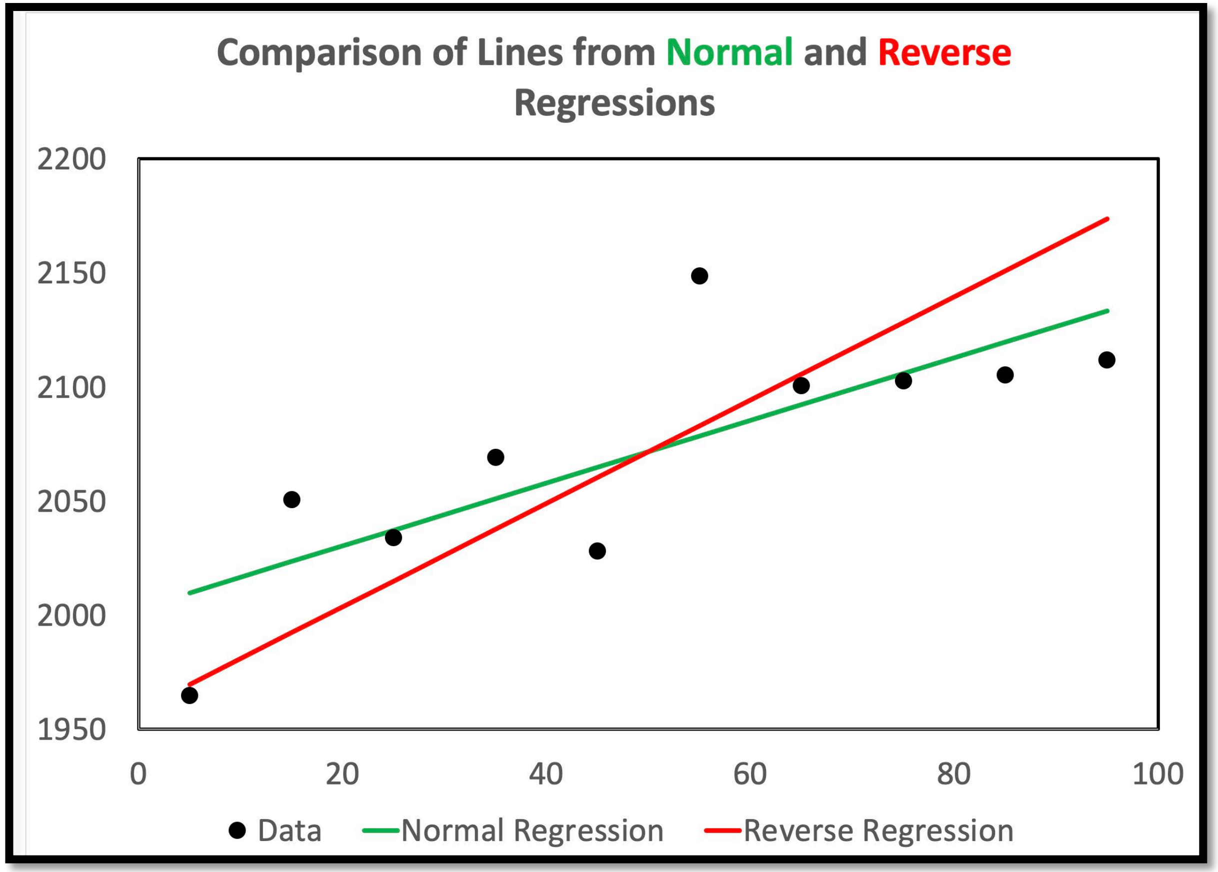


THE ANSWER IS THAT YOU MAY HAVE BETTER RESULTS:

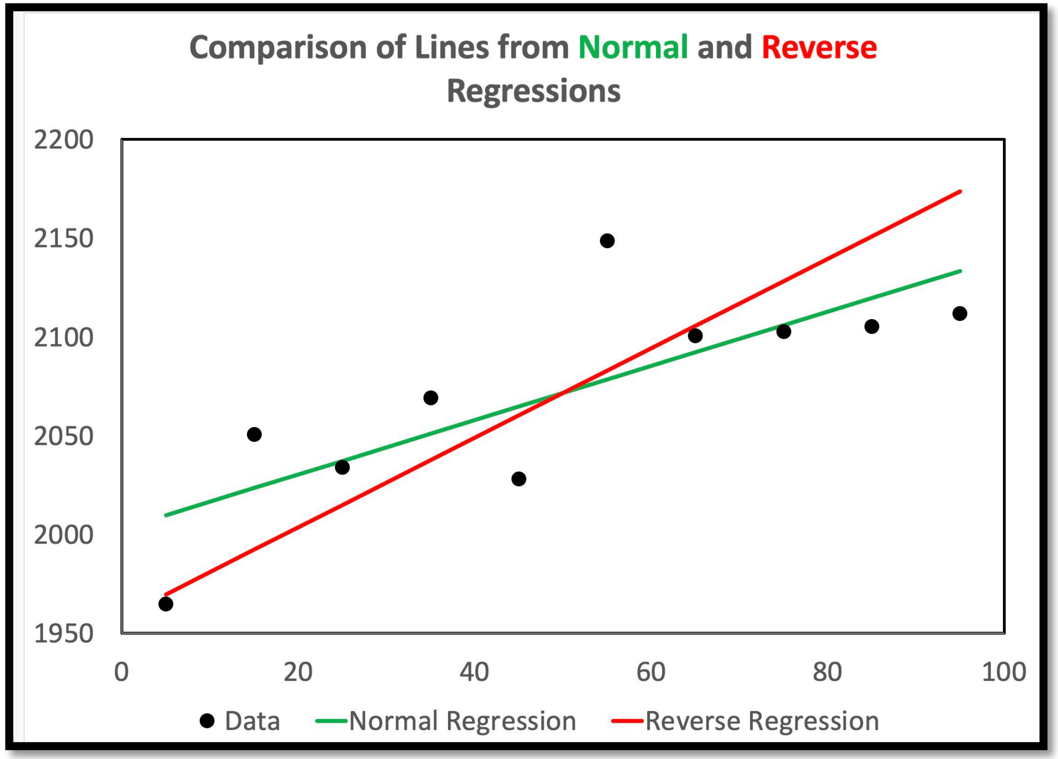
- With another method simply by making different calculations
- **With modern computers practically no effort at all**

What Makes Understanding **Why The Calibration Problem Really Is A Problem** Difficult?

- It is not obvious to most biologists that:
  - The line calculated is dependent on assumptions made
  - **ASSUMPTION OF y=*f* (X)**
    - **$Y=b_1(x) + b_0$**
  - **ASSUMPTION OF x=*f* (Y)**
    - **$Y = (x - b_0) / b_1$**
  - Both have exactly the same r^2



What Makes Understanding **Why The Calibration Problem Really Is A Problem** Difficult?



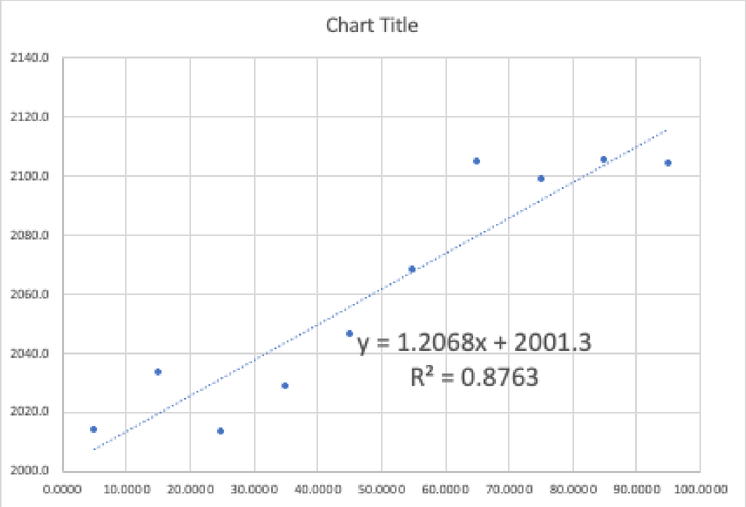
- It seems obvious that the equation with x as the independent variable can be inverted to find the value of x from y
- But when x is calculated directly from y, a different equation should be used!!
- Can it be appropriate to calculate variation for a variable that is believed to be without variation?

TRY THIS AT HOME:

IF EXCEL IS USED TO CALCULATE THE NORMAL AND REVERSE REGRESSIONS, THE RESULTING LINES ARE NOT THE SAME UNLESS R^2=1.000000…

y=*f* (x)

Standard or Calibration Curve		Normal Model	Calcs from XL
	b_1=	1.2068	1.20679
	b_0=	2001.3108	2001.311
	R^2=	0.8763	0.876344
SD about Regression	$s_{x/y}$=	14.5574	
SD of Calibration Slope	s_{b1}=	0.1603	
SD of Calibration Intercept	s_{b0}=	9.2417	

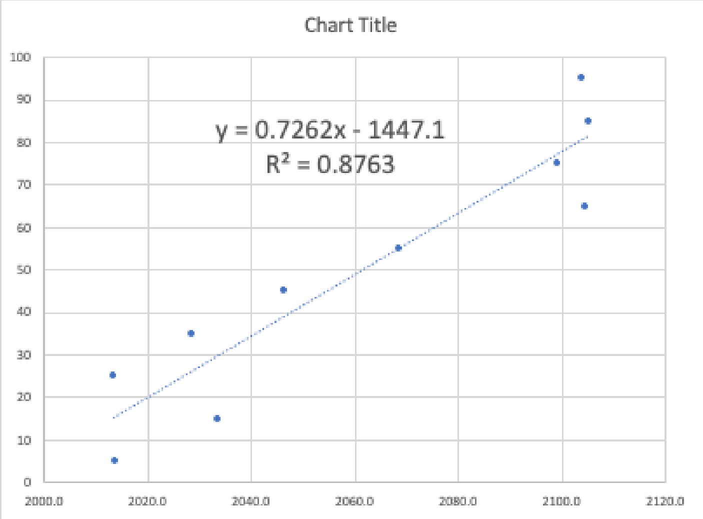


Calibration Data

Obs.	C_{std} x_i	S_{std} y_i
1	5.0000	2013.7
2	15.0000	2033.6
3	25.0000	2013.5
4	35.0000	2028.4
5	45.0000	2046.2
6	55.0000	2068.4
7	65.0000	2104.6
8	75.0000	2099.0
9	85.0000	2105.2
10	95.0000	2103.9
	Sum	Sum
	500	20617
	Avg	Avg
	50	2061.650

x=*f* (y)

Standard or Calibration Curve		Normal Model	Calcs from XL
	b_1=	0.7262	0.72618
	b_0=	-1447.1245	-1447.12
	R^2=	0.8763	0.87634
SD about Regression	$s_{x/y}$=	4963.3284	
SD of Calibration Slope	s_{b1}=	42.3889	
SD of Calibration Intercept	s_{b0}=	87405.0975	



Calibration Data

Obs.	C_{std} x_i	S_{std} y_i
1	2013.7	5
2	2033.6	15
3	2013.5	25
4	2028.4	35
5	2046.2	45
6	2068.4	55
7	2104.6	65
8	2099.0	75
9	2105.2	85
10	2103.9	95
	Sum	Sum
	20616.503	500.000
	Avg	Avg
	2061.6503	50.000

HOW ARE CALIBRATION OR STANDARD CURVES TYPICALLY EVALUATED?

- The **COUNTER-INTUITIVE** method uses one-way ANOVA and multiple range tests to determine which standards the test samples are not different from.
- The **INTUITIVE** method uses classical (or normal, or standard) regression in the form of $y=f(x)$ and is inverted $x=(y-b_0)/b_1$ to find variation in x in test samples.
- The **SOPHISTIC** method uses reverse regression in the form of $x=g(y)$ and $x=b_1(y)-b_0$ to find variation in x in test samples.
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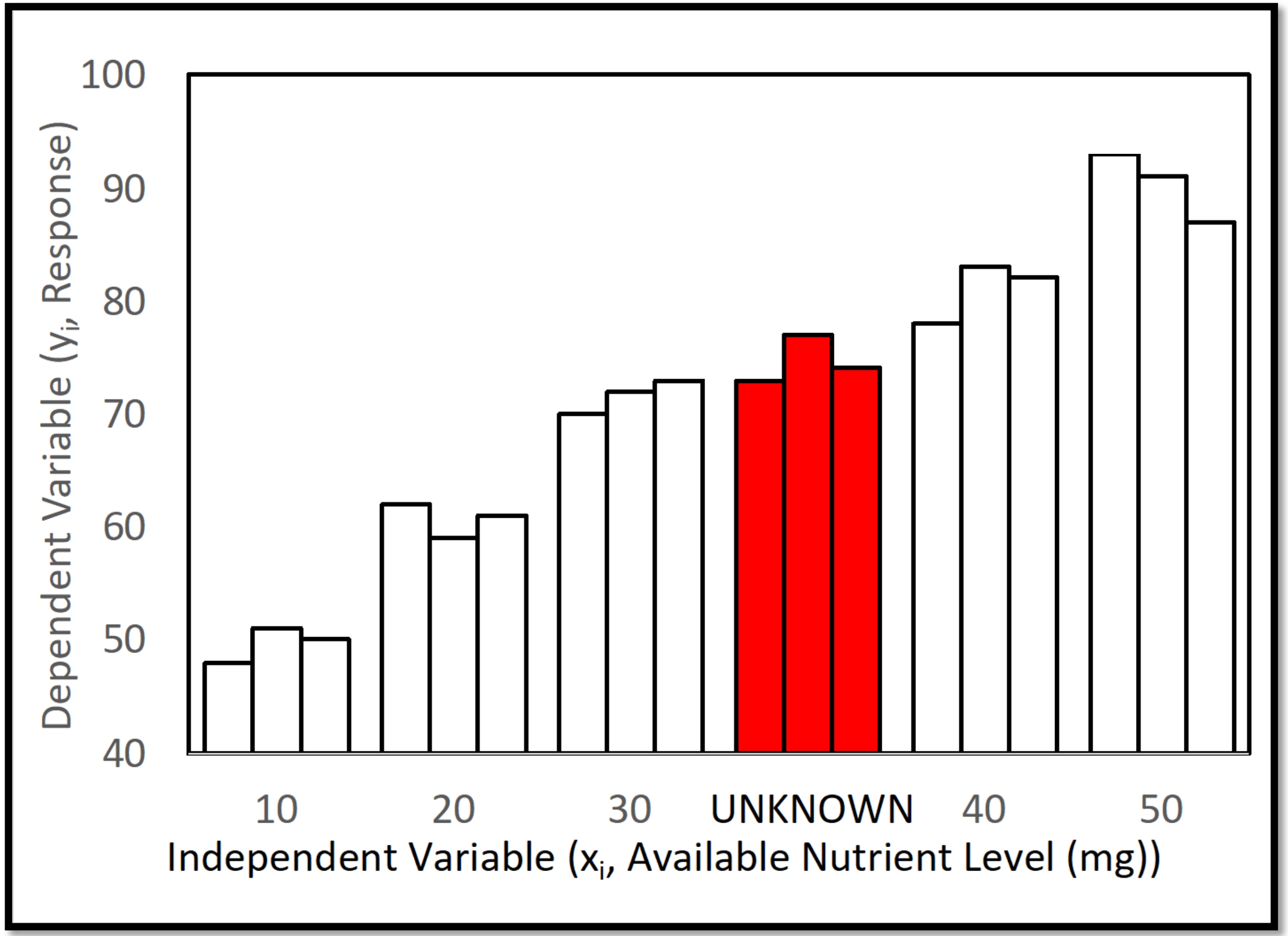
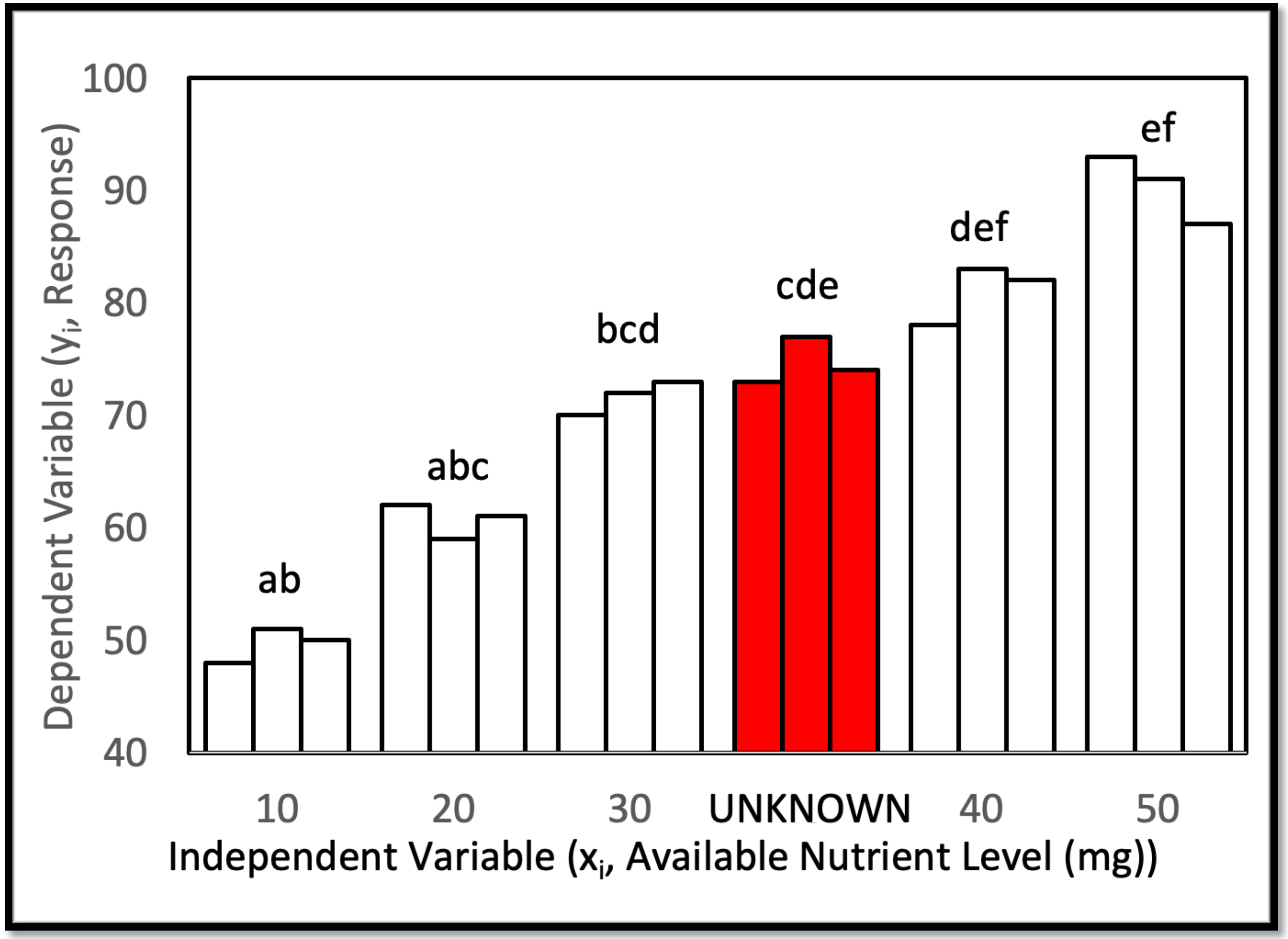
Determining The Value Of Some Unknown Property Of A Sample

- Compare the “unknow” sample to samples of known composition
- Conduct an experiment and get responses from the TEST and known STANDARD samples



Determining The Value Of Some Unknown Property Of A Sample

- First thought may be to compare values of known and unknown samples using t-tests or multiple-range tests



- The results do not identify the content of the unknown sample.
- The results only tell what the sample is not different from.
- So this approach is counter-intuitive. It does not answer the question of what is the content of the test or "unknown" sample.

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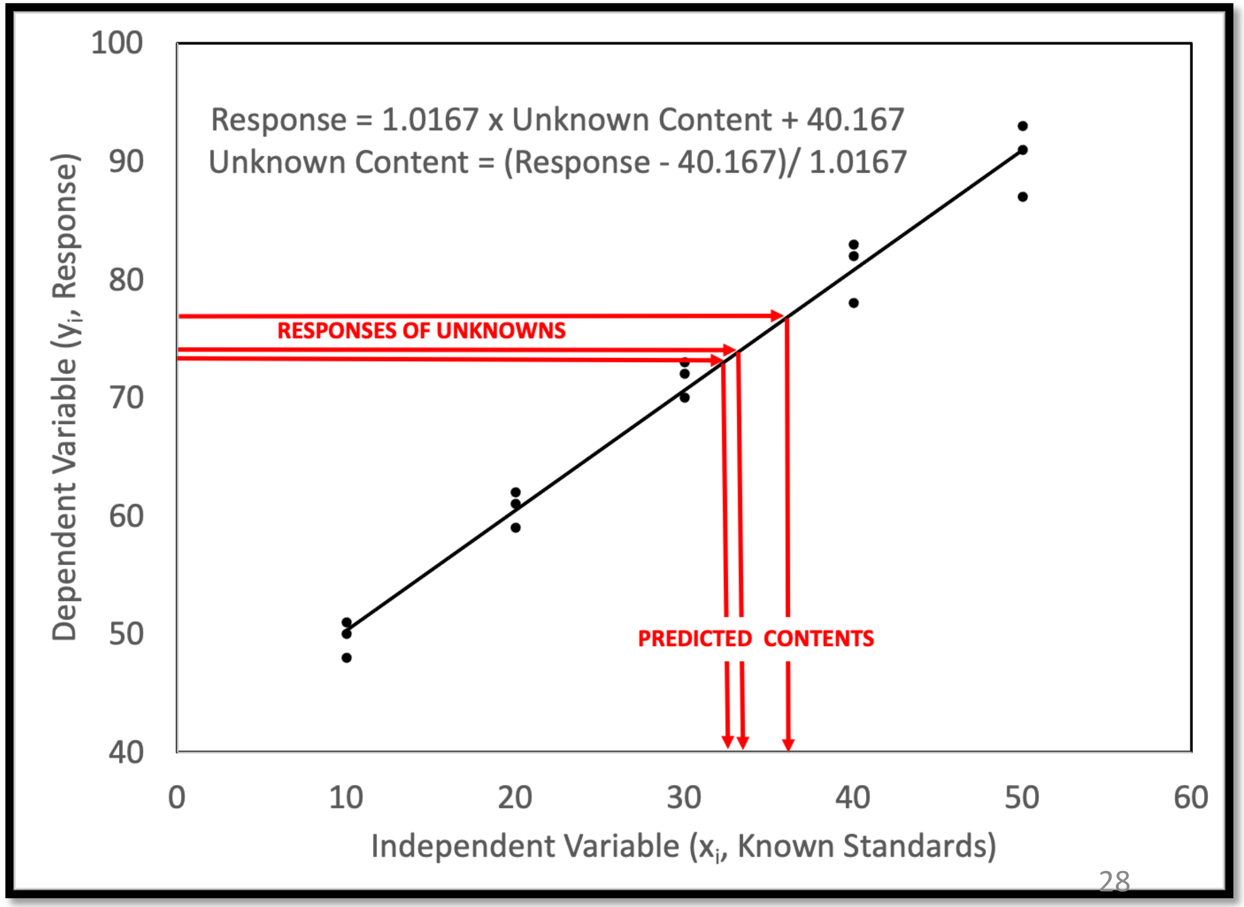
DETERMINING THE VALUE OF SOME UNKNOWN PROPERTY OF A SAMPLE – NORMAL REGRESSION & INVERSE PREDICTION

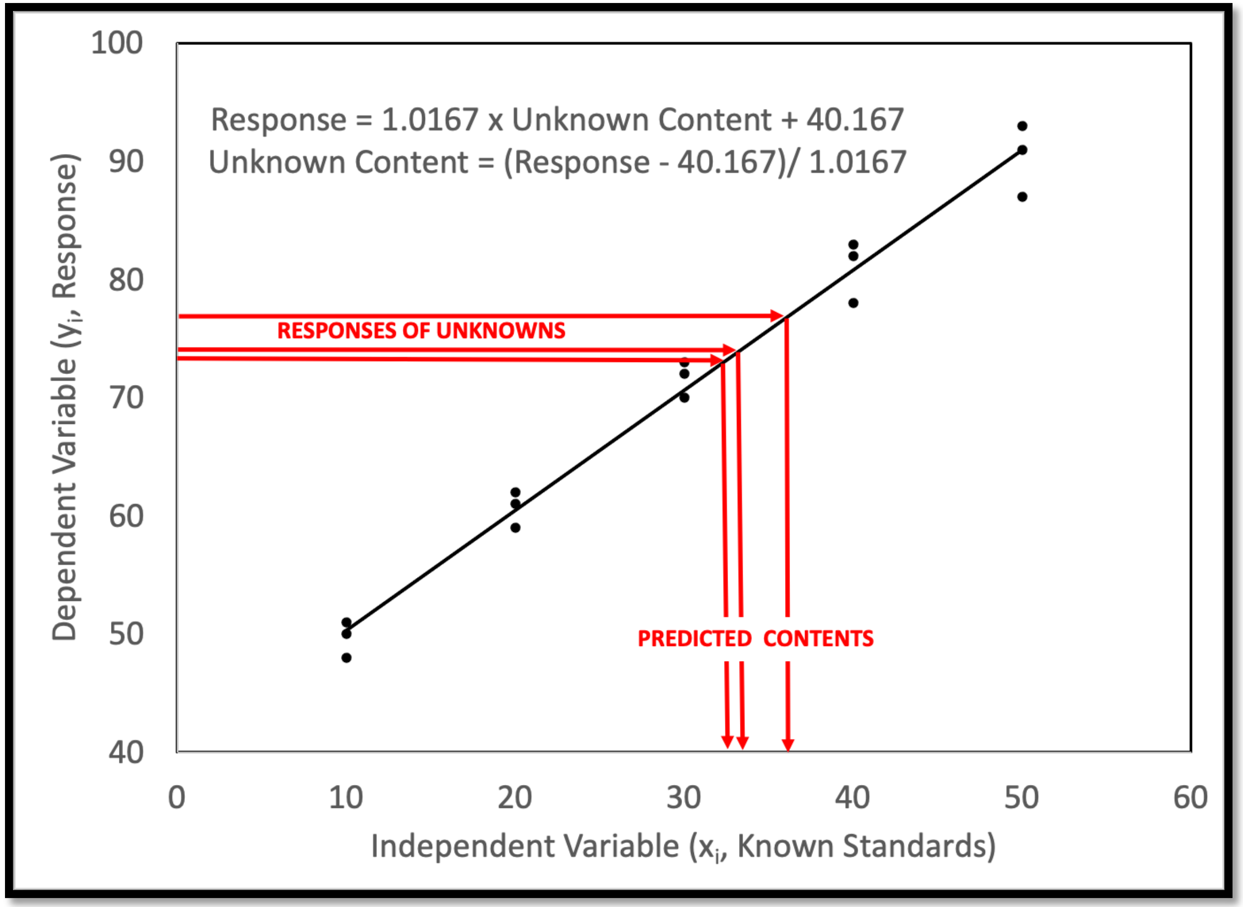
- **This approach is to compare the responses of the test and standard samples using simple linear regression**

- The results do identify the content of the test sample.
- If there are replications of the test sample responses, an SD of the amount of unknown in the sample can be calculated
- So this approach is intuitive. It answers the question of what is the content of the "unknown" in the sample



DETERMINING THE VALUE OF SOME UNKNOWN PROPERTY OF A SAMPLE - NORMAL REGRESSION & INVERSE PREDICTION

- **THIS APPROACH MAY BE INTUITIVE: COMPARE THE RESPONSES OF THE TEST AND STANDARD SAMPLES USING SIMPLE LINEAR REGRESSION**



- This process is called Inverse Prediction
- With regression,
  - $y=f(x)$, $y=b_1x+b_0$
- With Inverse prediction, the equation is rearranged to get $x=f(y)$, $x=(y-b_0)/b_1$

ASSUMPTIONS OF INVERSE PREDICTION?

ASSUMPTIONS OF REGRESSION

- Y is dependent on X
  - Y=*f* (X)
- X values are known precisely
  - There is no variation in X values
- There is variation in Y

ASSUMPTIONS OF INVERSE PREDICTION

- X is dependent on Y
  - X=*g* (Y)
  - Y values are known precisely
  - There is variation in X values
  - There is no variation in Y

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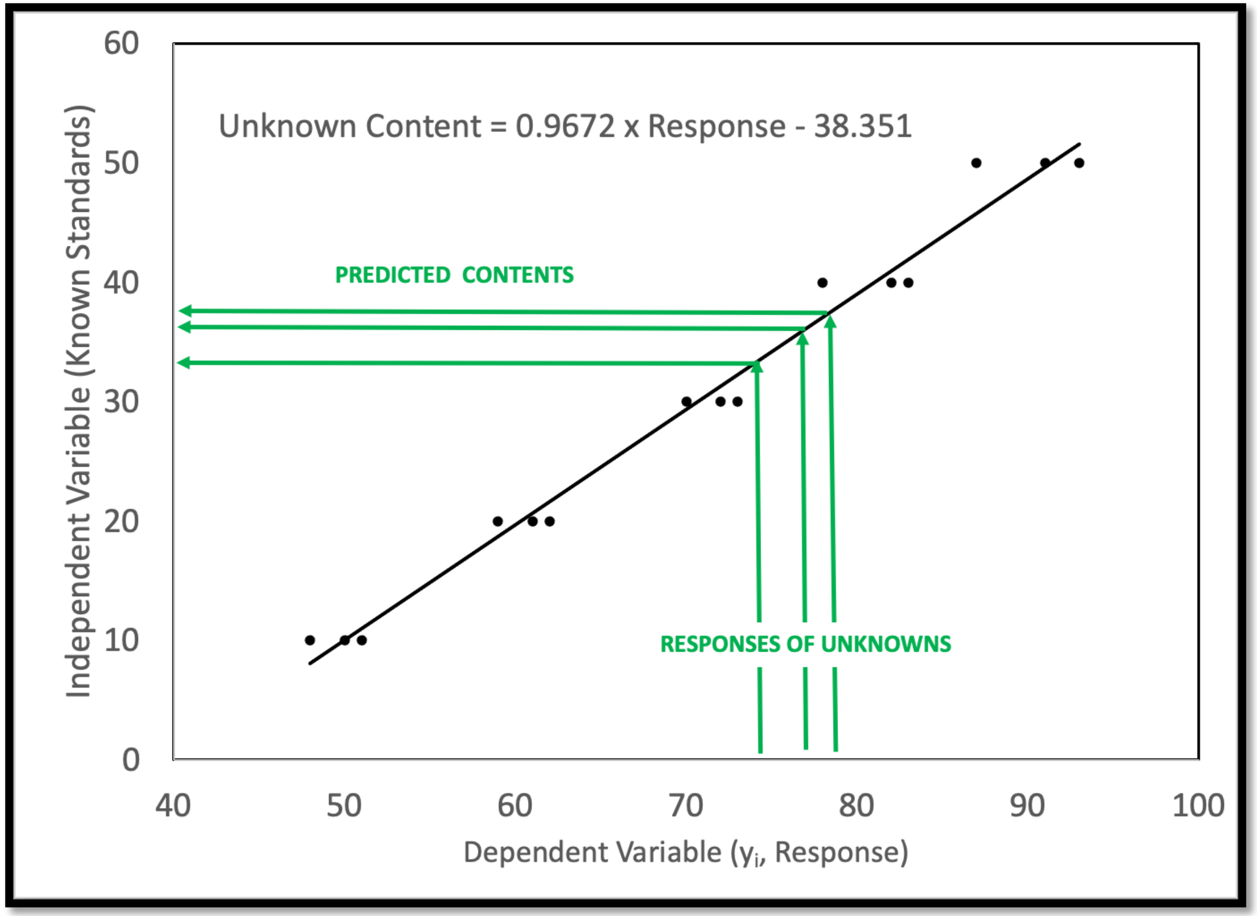
REVERSE REGRESSION

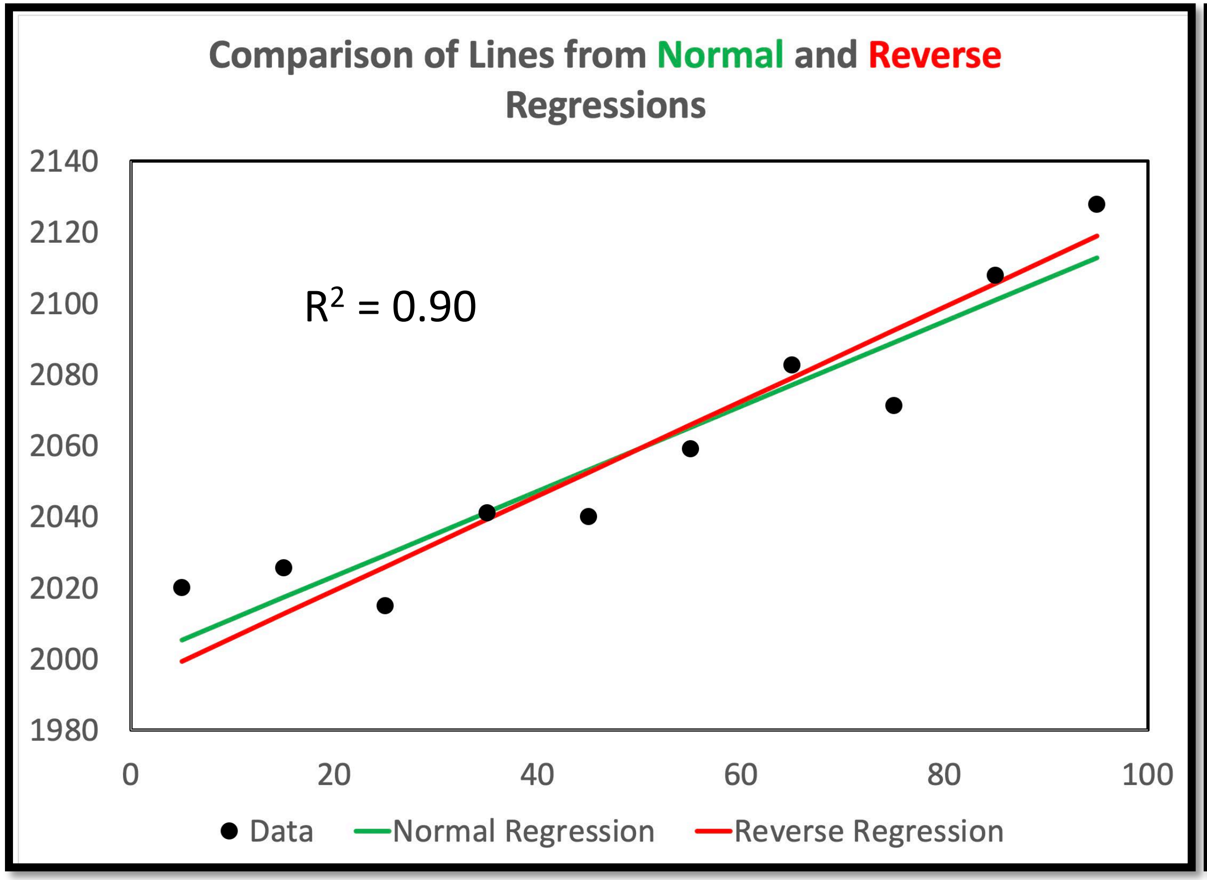
- Switches X and Y axes
- Calculate $X=b_1(Y)-b_0$
- Estimate X of test samples directly from equation
- More problems than intuitive
  - No variation in curve
  - 0 degrees of freedom for each test sample
  - Uses wrong equation for prediction

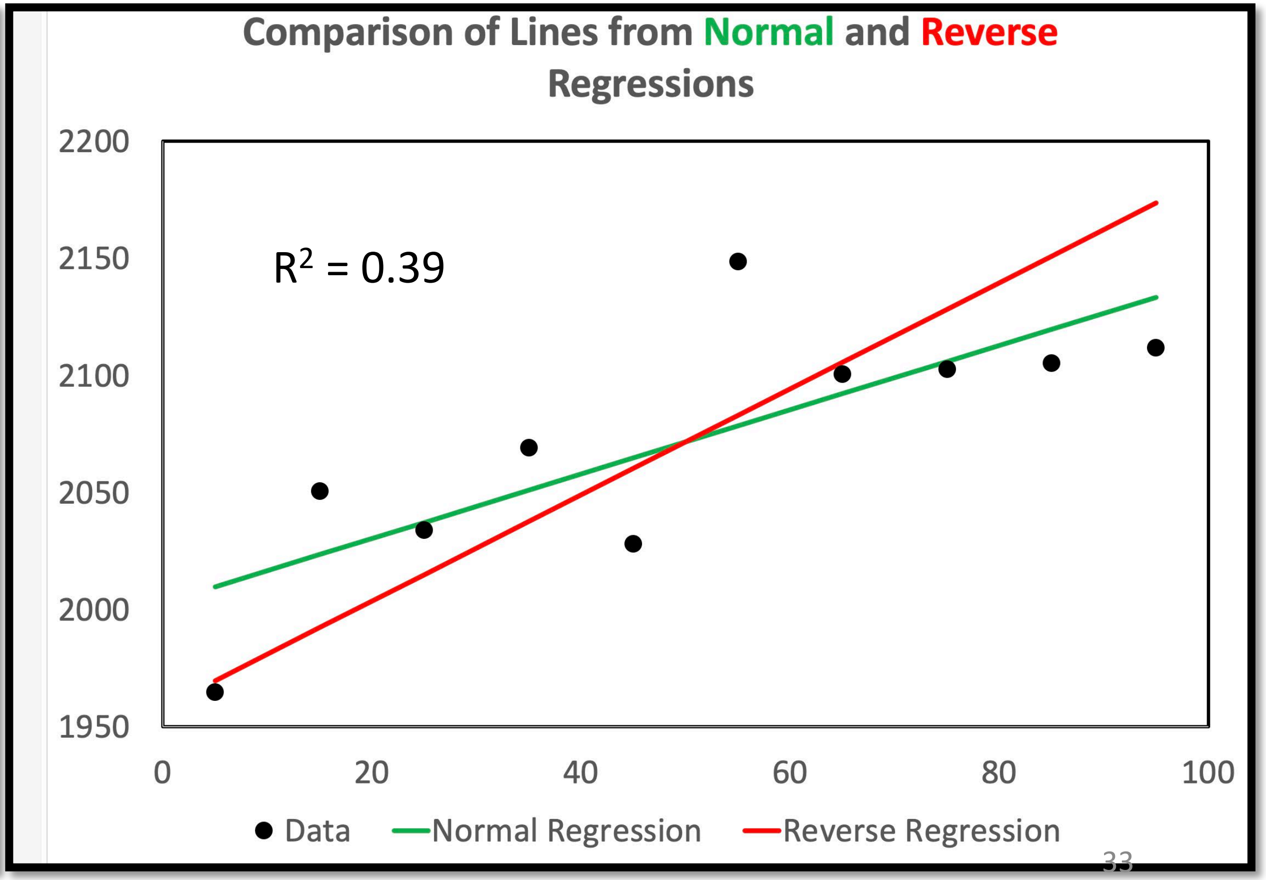


Reverse Regression **Reality Check**

Uses wrong equation for prediction

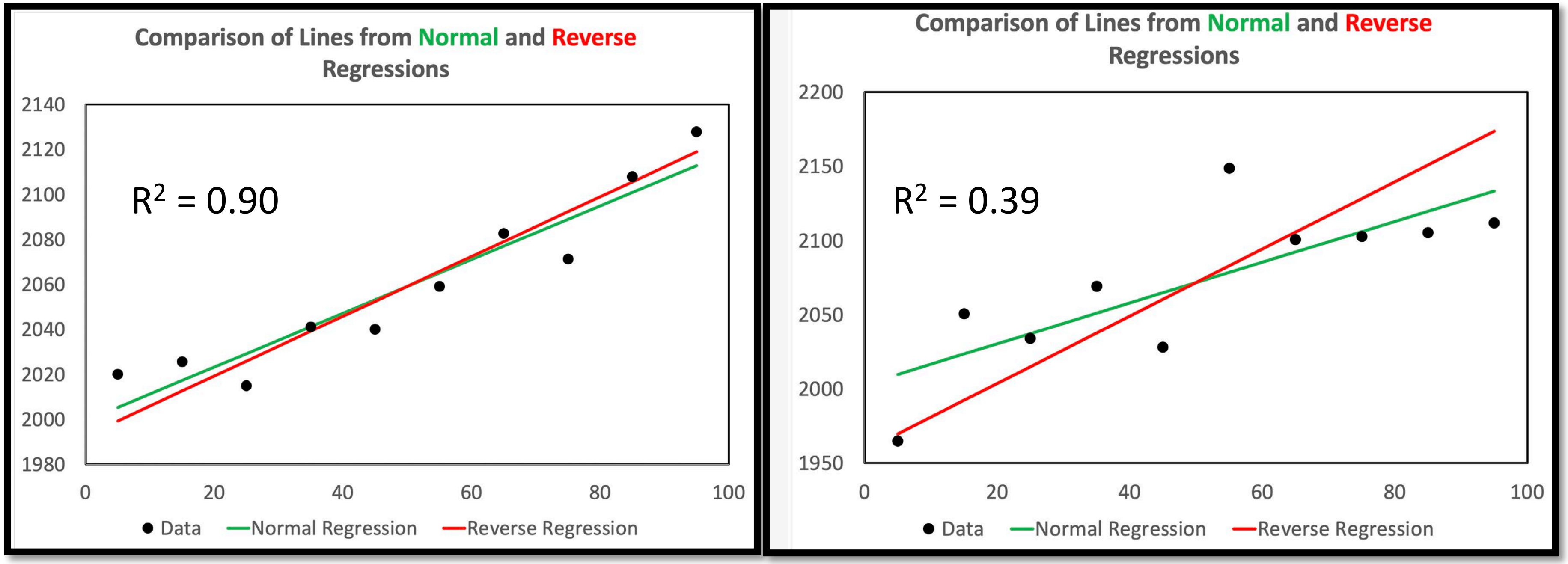
- Degree of wrongness depends on R^2 of line
- With R^2>0.95, there is very little difference in lines





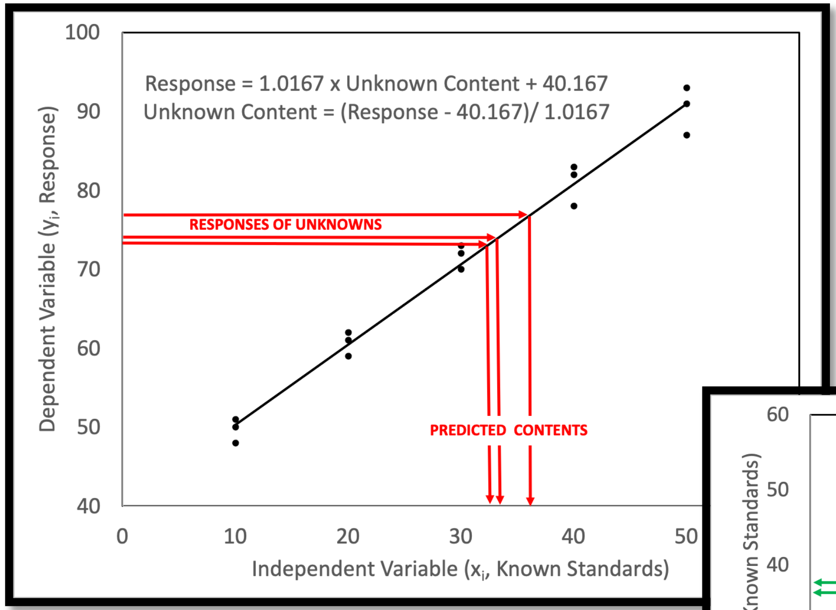
Reverse Regression Reality Check

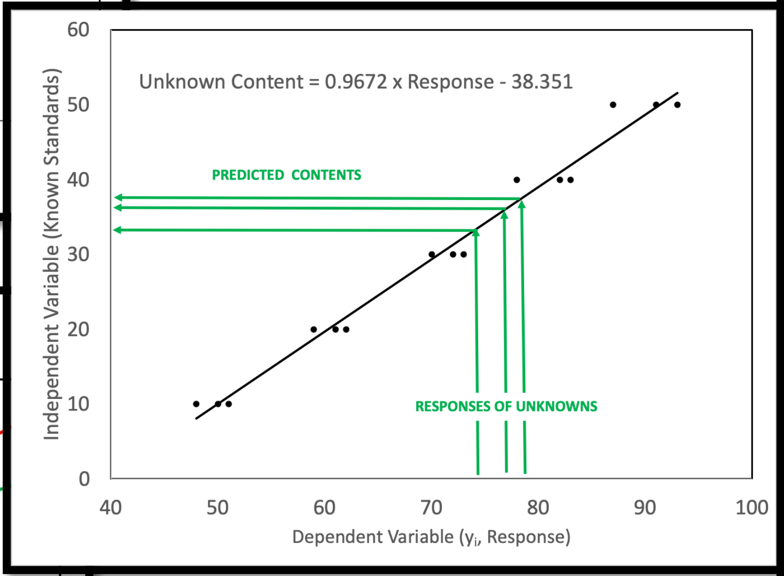
- Understood: ”not significantly different” does not mean “the same as”
- **Significantly different from wrong does not mean right**
- The sophistic method gives the wrong mean, the c. I. Is irrelevant!!!

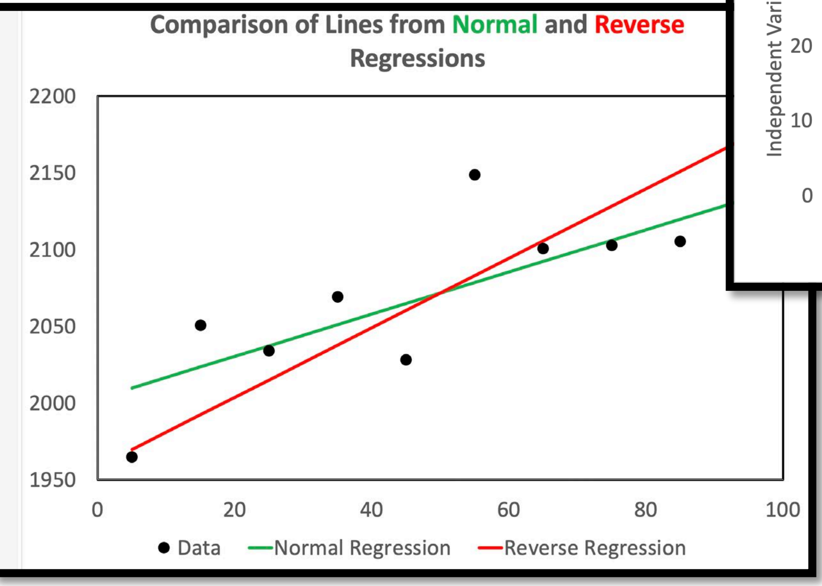


- The mean estimate from the sophostic model may be close, but except for one point it is still **always** wrong

Inverse Prediction & The Reverse Regression: Both Techniques Are Problematic – Assumptions Not True







- Pretend standard curve has no C.I.
- 0 degrees of freedom predictions
- Overestimate variation
- A method for estimating the confidence interval of test samples should be based on observations and theories, not concepts known not to be true.

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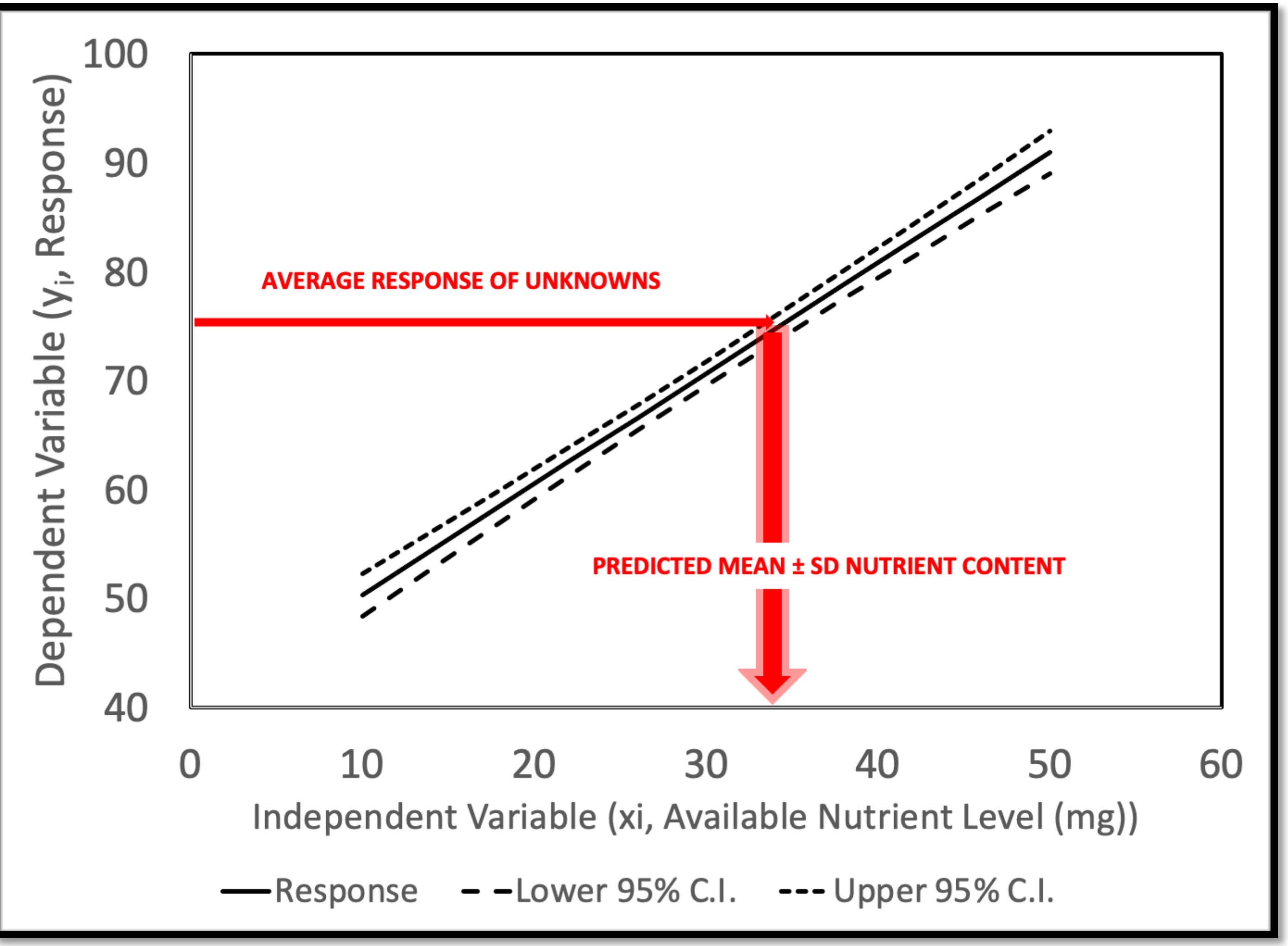
To Estimate The Variation In X From An Observed Y Requires Abductive Reasoning

- Abductive models make best ~~guesses~~ estimates of the C.I. For what X really is



- Abductive reasoning uses known principles and observations to estimate the SD of unknown samples.
- Abductive reasoning can account for changes in the confidence interval near the average X values and extreme X values.
- The mean X values are the same with intuitive and abductive models.
- Abductive models can show the increase in uncertainty in X predictions at the extremes of the calibration curve.
  - The intuitive model can not.

"**Graybill's Equation**" Includes Variation From Both **Uncertainty** In The **Line** And In The **Unknown**

$$s_0 = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}}$$

and

$$s_{x/y} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}}$$

- S_0 = standard deviation of the estimated x_0 from an unknown response, y_0
- B is the slope of the line
- M = number of replicates of (y_0)
- N = number of responses (y_i) to the standards (x_i)
- Y_0 is the average of the unknown samples
- $S_{y/x}$ = standard error of the regression line

$$s_0 = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{b^2\sum_i(x_i-\bar{x})^2}}$$

- Graybill's abductive method is useful for:
  1. Estimating experimental power

     Influence of changing replicates or standard levels on s_0
  2. Quality control

     Influence of difference from $\bar{X}$ on magnitude of the confidence interval

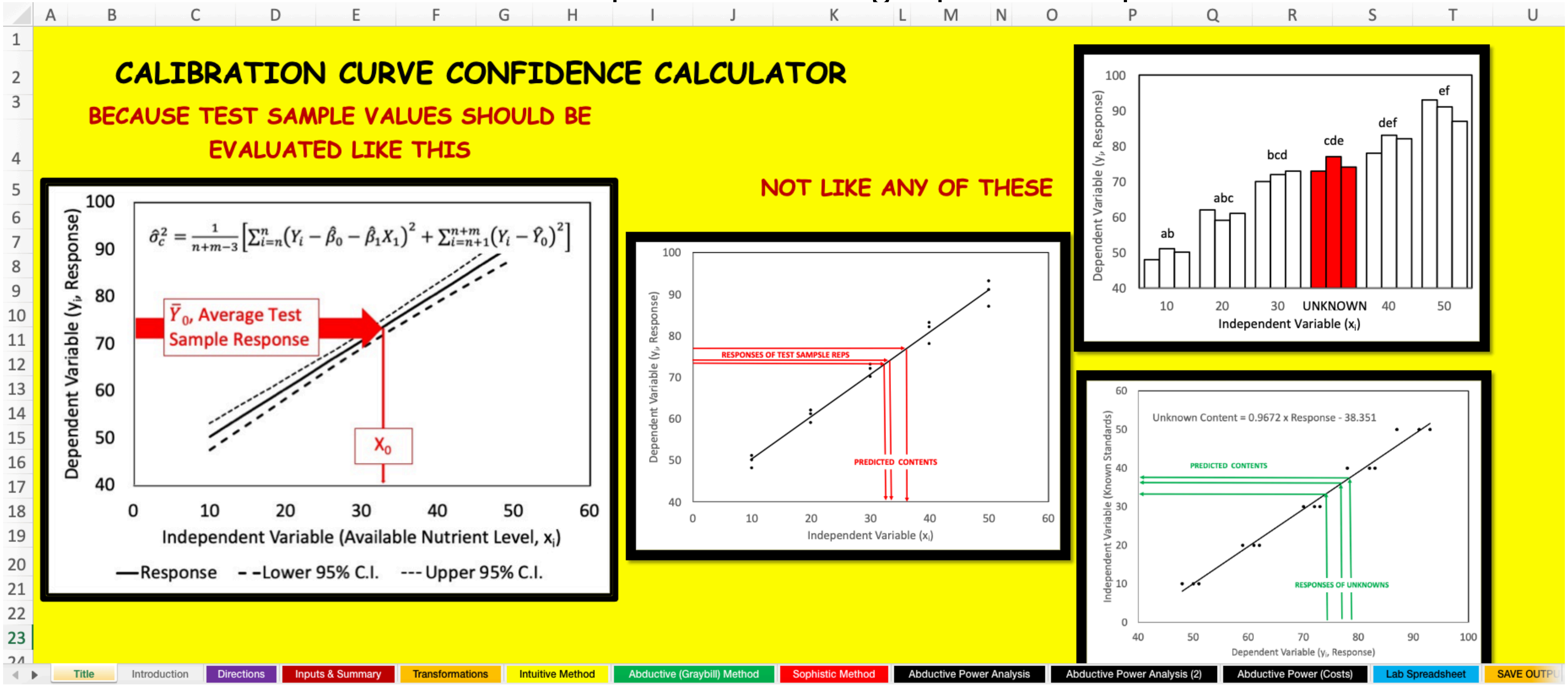
How Are Calibration Or Standard Curves Typically Evaluated?

And now to show some comparisons of actual data

- The **counter-intuitive** method uses one-way ANOVA and multiple range tests to determine which standards the test samples are not different from.
- The **intuitive** method uses classical (or normal, or standard) regression in the form of $y=f(x)$ and inverted $x=(y-b_0)/b_1$ to find variation in x in test samples
- The **sophistic** method uses reverse regression in the form of $x=f(y)$ and $x=b_1(y)-b_0$ to find variation in x in test samples
- **Abductive** methods use standard regression in the form of $y=f(x)$ and $x=(y-b_0)/b_1$ to find the mean value for x. They use equations based on observation and theory to find variation in x in test samples

A Microsoft Excel Workbook Written To Make Calculations And Comparisons

It can also be helpful in estimating experimental power



ENTER THE VALUES OF YOUR STANDARD CURVE IN THE GREEN CELLS

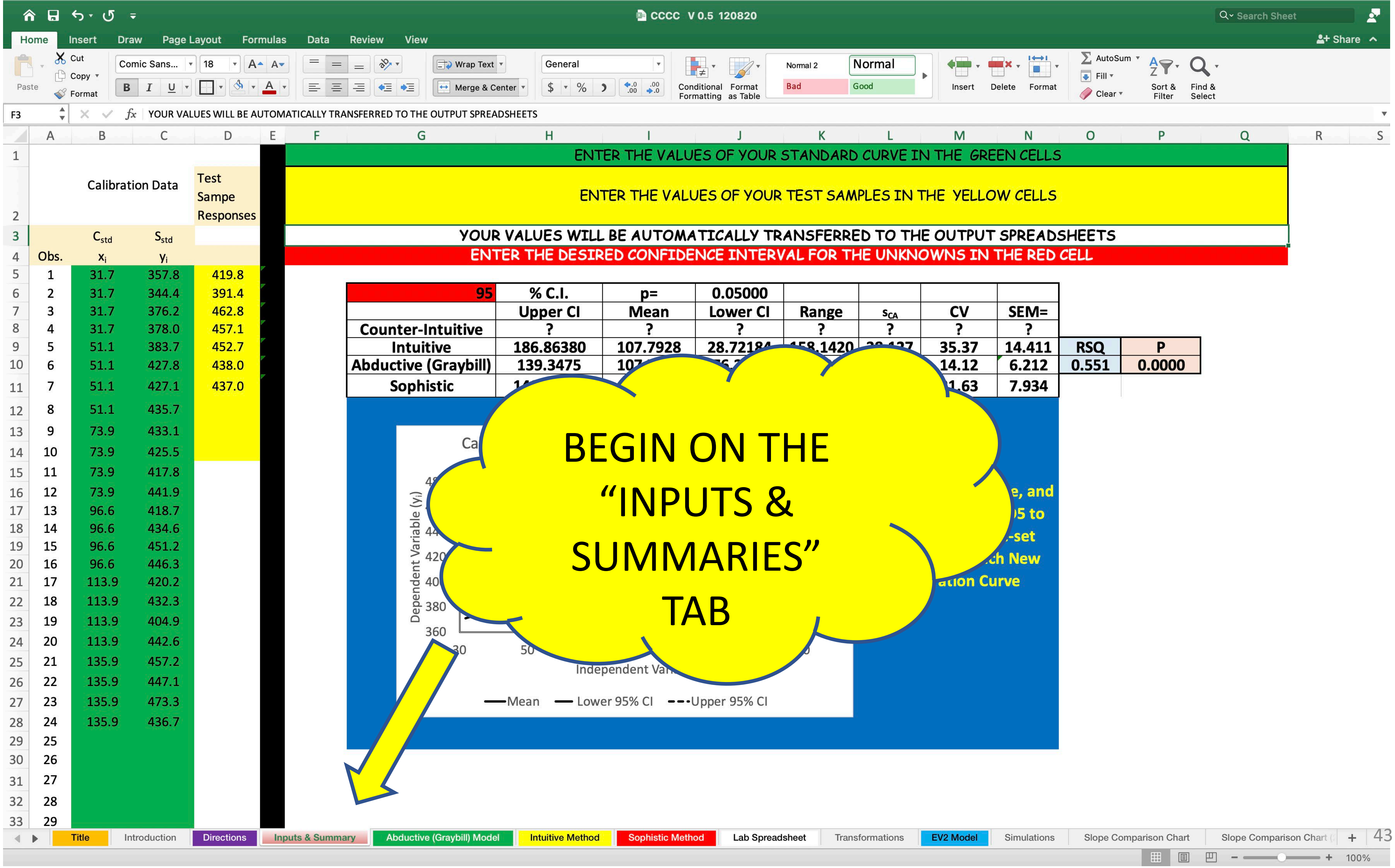
ENTER THE VALUES OF YOUR TEST SAMPLES IN ...

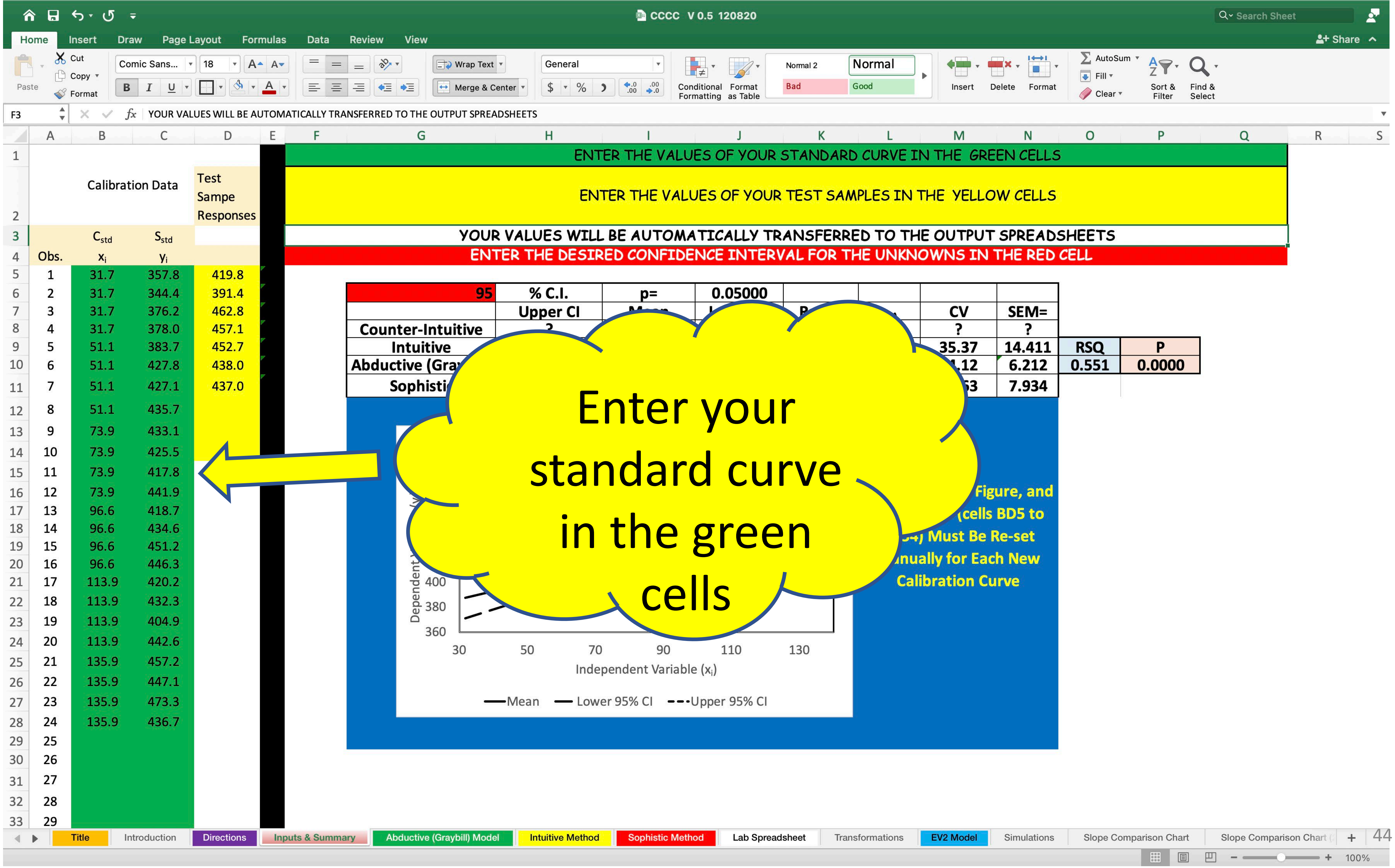
Calibration Data

Obs.	C_{std} x_i	S_{std} y_i	Test Sampe Responses
1	31.7	357.8	419.8
2	31.7	344.4	391.4
3	31.7	376.2	462.8
4	31.7	378.0	457.1
5	51.1	383.7	452.7
6	51.1	427.8	438.0
7	51.1	427.1	437.0
8	51.1	435.7	
9	73.9	433.1	
10	73.9	425.5	
11	73.9	417.8	
12	73.9	441.9	
13	96.6	418.7	
14	96.6	434.6	
15	96.6	451.2	
16	96.6	446.3	
17	113.9	420.2	
18	113.9	432.3	
19	113.9	404.9	
20	113.9	442.6	
21	135.9	457.2	
22	135.9	447.1	
23	135.9	473.3	
24	135.9	436.7	
25			
26			
27			
28			
29			

An example: the tibia zinc of chicks

- Standard curve from feeding different levels of a highly available zinc source
- Test sample from feeding a standard source of zinc

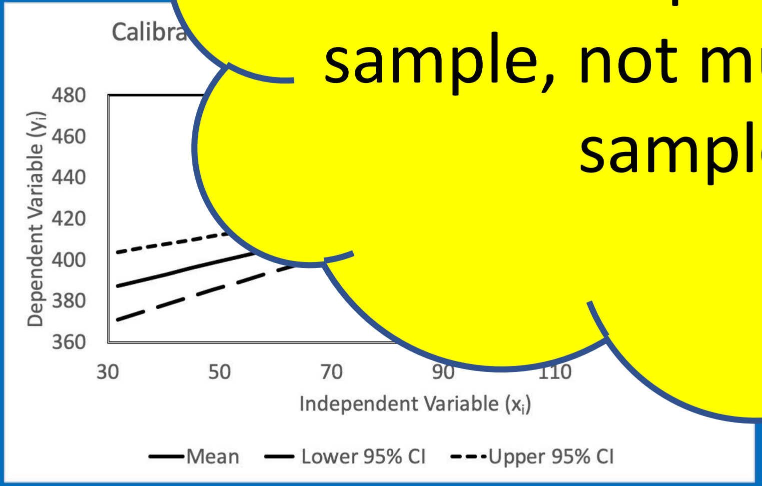

CCCC V 0.5 120820
YOUR VALUES WILL BE AUTOMATICALLY TRANSFERRED TO THE OUTPUT SPREADSHEETS
ENTER THE VALUES OF YOUR STANDARD CURVE IN THE GREEN CELLS
ENTER THE VALUES OF YOUR TEST SAMPLES IN THE YELLOW CELLS
ENTER THE DESIRED CONFIDENCE INTERVAL FOR THE UNKNOWNS IN THE RED CELL
Calibration Data
Test Sampe Responses
Obs.
95
% C.I.
p=
0.05000
Upper CI
Mean
Lower CI
Range
CV
SEM=
Counter-Intuitive
Intuitive
Abductive (Graybill)
Sophistic
RSQ
P
0.551
0.0000
BEGIN ON THE "INPUTS & SUMMARIES" TAB
Mean
Lower 95% CI
Upper 95% CI
Title
Introduction
Directions
Inputs & Summary
Abductive (Graybill) Model
Intuitive Method
Sophistic Method
Lab Spreadsheet
Transformations
EV2 Model
Simulations
Slope Comparison Chart

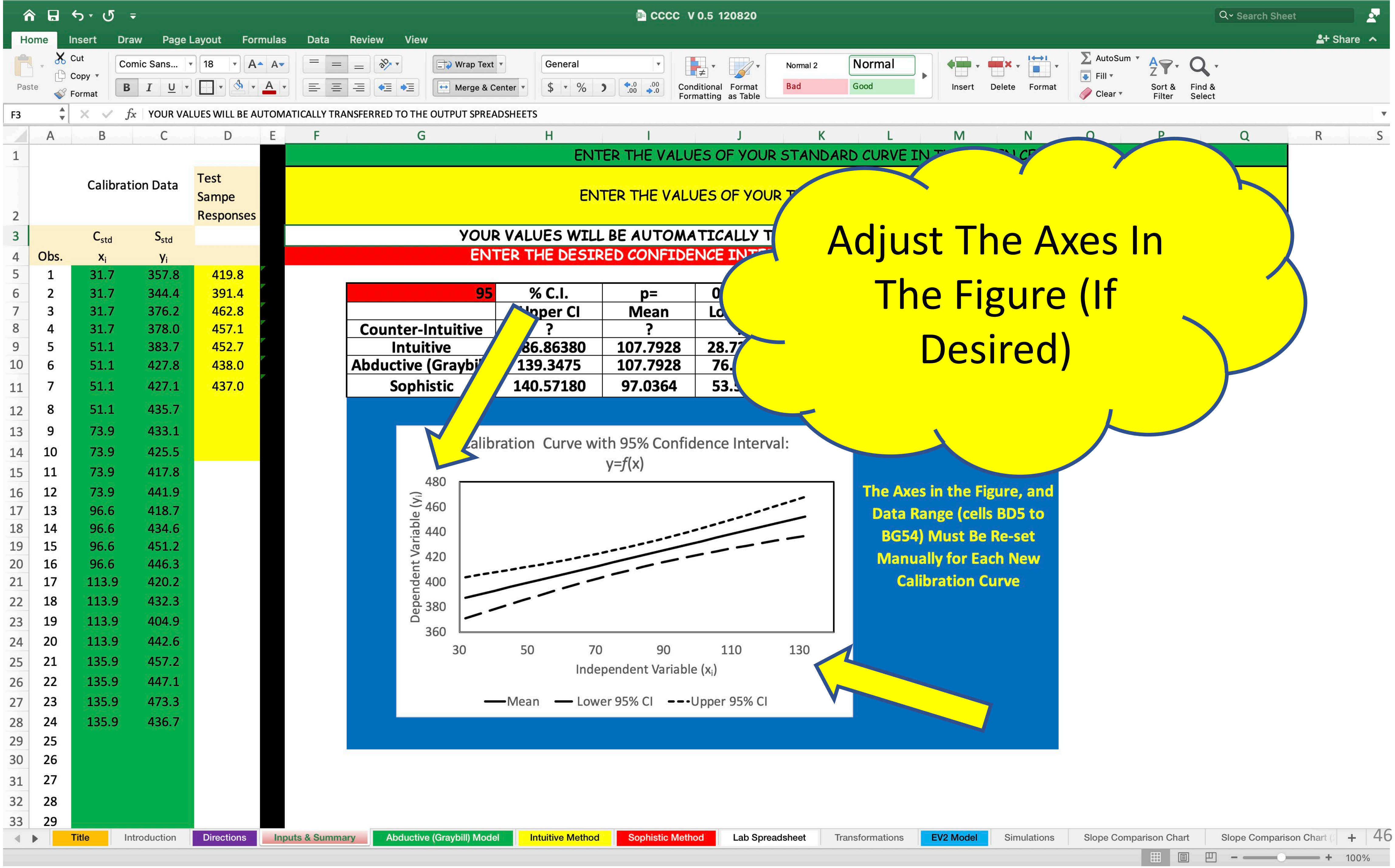


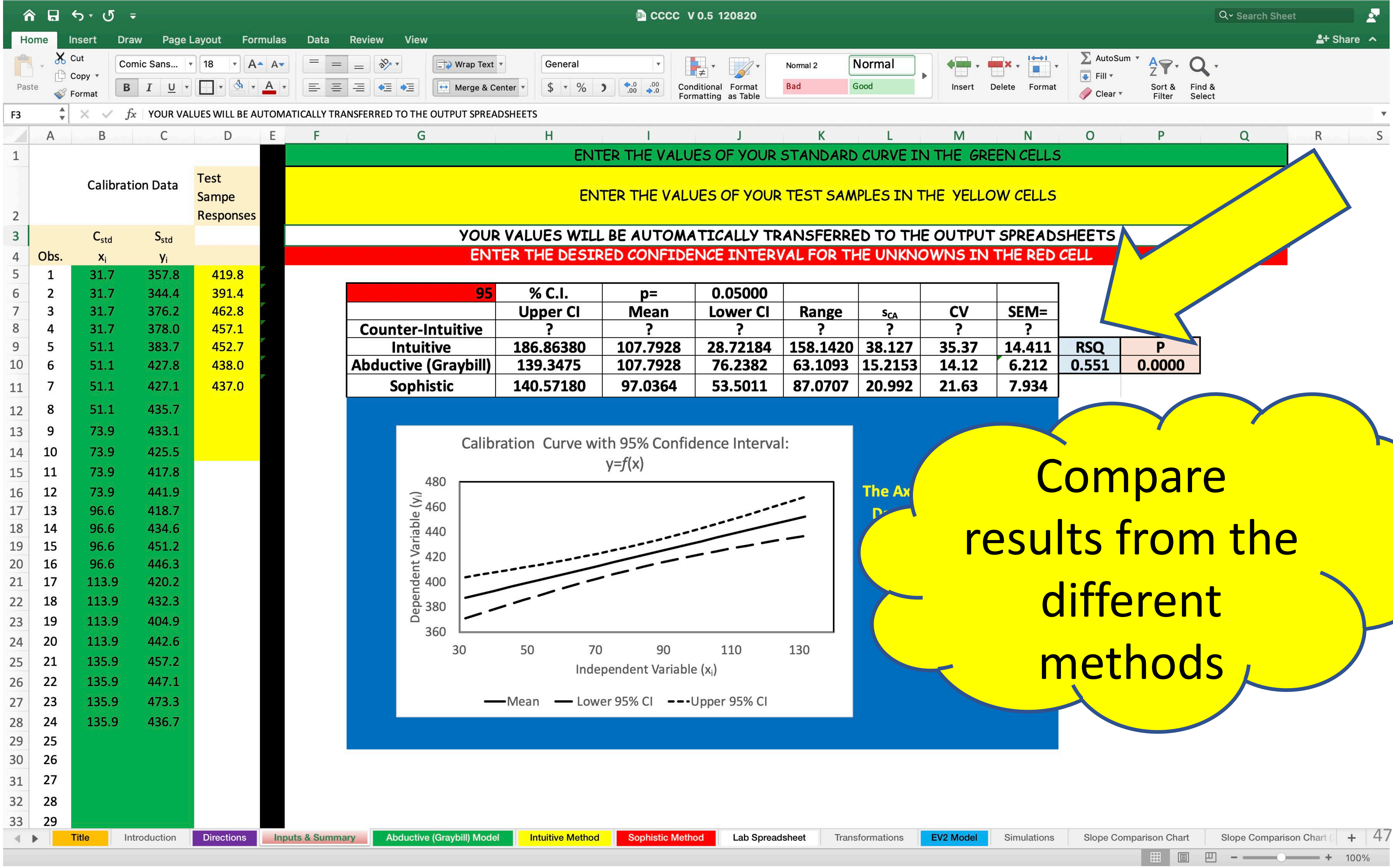
Obs.	Calibration Data C_{std} x_i	S_{std} y_i	Test Sampe Responses
1	31.7	357.8	419.8
2	31.7	344.4	391.4
3	31.7	376.2	462.8
4	31.7	378.0	457.1
5	51.1	383.7	452.7
6	51.1	427.8	438.0
7	51.1	427.1	437.0
8	51.1	435.7	
9	73.9	433.1	
10	73.9	425.5	
11	73.9	417.8	
12	73.9	441.9	
13	96.6	418.7	
14	96.6	434.6	
15	96.6	451.2	
16	96.6	446.3	
17	113.9	420.2	
18	113.9	432.3	
19	113.9	404.9	
20	113.9	442.6	
21	135.9	457.2	
22	135.9	447.1	
23	135.9	473.3	
24	135.9	436.7	
25			
26			
27			
28			
29			

Enter your test sample results in the yellow cells These are replicates of one sample, not multiple test samples

Obs.	Calibration Data C_{std} x_i	S_{std} y_i	Test Sampe Responses
1	31.7	357.8	419.8
2	31.7	344.4	391.4
3	31.7	376.2	462.8
4	31.7	378.0	457.1
5	51.1	383.7	452.7
6	51.1	427.8	438.0
7	51.1	427.1	437.0
8	51.1	435.7	
9	73.9	433.1	
10	73.9	425.5	
11	73.9	417.8	
12	73.9	441.9	
13	96.6	418.7	
14	96.6	434.6	
15	96.6	451.2	
16	96.6	446.3	
17	113.9	420.2	
18	113.9	432.3	
19	113.9	404.9	
20	113.9	442.6	
21	135.9	457.2	
22	135.9	447.1	
23	135.9	473.3	
24	135.9	436.7	
25			
26			
27			
28			
29			




CCCC V 0.5 120820
YOUR VALUES WILL BE AUTOMATICALLY TRANSFERRED TO THE OUTPUT SPREADSHEETS
Calibration Data
Test Sampe Responses
ENTER THE VALUES OF YOUR STANDARD CURVE IN
ENTER THE VALUES OF YOUR
YOUR VALUES WILL BE AUTOMATICALLY T
ENTER THE DESIRED CONFIDENCE INT
Adjust The Axes In The Figure (If Desired)
Calibration Curve with 95% Confidence Interval: y=f(x)
Dependent Variable (yi)
Independent Variable (xi)
Mean
Lower 95% CI
Upper 95% CI
The Axes in the Figure, and Data Range (cells BD5 to BG54) Must Be Re-set Manually for Each New Calibration Curve
Title
Introduction
Directions
Inputs & Summary
Abductive (Graybill) Model
Intuitive Method
Sophistic Method
Lab Spreadsheet
Transformations
EV2 Model
Simulations
Slope Comparison Chart


CCCC V 0.5 120820
YOUR VALUES WILL BE AUTOMATICALLY TRANSFERRED TO THE OUTPUT SPREADSHEETS
Calibration Data
Test Sampe Responses
Obs.
C_{std} x_i
S_{std} y_i
1 31.7 357.8 419.8
2 31.7 344.4 391.4
3 31.7 376.2 462.8
4 31.7 378.0 457.1
5 51.1 383.7 452.7
6 51.1 427.8 438.0
7 51.1 427.1 437.0
8 51.1 435.7
9 73.9 433.1
10 73.9 425.5
11 73.9 417.8
12 73.9 441.9
13 96.6 418.7
14 96.6 434.6
15 96.6 451.2
16 96.6 446.3
17 113.9 420.2
18 113.9 432.3
19 113.9 404.9
20 113.9 442.6
21 135.9 457.2
22 135.9 447.1
23 135.9 473.3
24 135.9 436.7
25
26
27
28
29
ENTER THE VALUES OF YOUR STANDARD CURVE IN THE GREEN CELLS
ENTER THE VALUES OF YOUR TEST SAMPLES IN THE YELLOW CELLS
YOUR VALUES WILL BE AUTOMATICALLY TRANSFERRED TO THE OUTPUT SPREADSHEETS
ENTER THE DESIRED CONFIDENCE INTERVAL FOR THE UNKNOWNS IN THE RED CELL
95 % C.I. p= 0.05000
Upper CI Mean Lower CI Range s_{CA} CV SEM=
Counter-Intuitive ? ? ? ? ? ? ?
Intuitive 186.86380 107.7928 28.72184 158.1420 38.127 35.37 14.411
Abductive (Graybill) 139.3475 107.7928 76.2382 63.1093 15.2153 14.12 6.212
Sophistic 140.57180 97.0364 53.5011 87.0707 20.992 21.63 7.934
RSQ P
0.551 0.0000
Calibration Curve with 95% Confidence Interval: y=f(x)
Dependent Variable (y_i)
Independent Variable (x_i)
Mean Lower 95% CI Upper 95% CI
Compare results from the different methods
Title Introduction Directions Inputs & Summary Abductive (Graybill) Model Intuitive Method Sophistic Method Lab Spreadsheet Transformations EV2 Model Simulations Slope Comparison Chart Slope Comparison Chart

95	% C.I.	p=	0.05000						
	Upper CI	Mean	Lower CI	Range	s_{CA}	CV	SEM=		
Counter-Intuitive	?	?	?	?	?	?	?		
Intuitive	186.86380	107.7928	28.72184	158.1420	38.127	35.37	14.411	RSQ	P
Abductive (Graybill)	139.3475	107.7928	76.2382	63.1093	15.2153	14.12	6.212	0.551	0.0000
Sophistic	140.57180	97.0364	53.5011	87.0707	20.992	21.63	7.934		

Mean values for intuitive and abductive models are the same

The confidence interval for the abductive method is much smaller

95	% C.I.	p=	0.05000						
	Upper CI	Mean	Lower CI	Range	s_{CA}	CV	SEM=		
Counter-Intuitive	?	?	?	?	?	?	?		
Intuitive	186.86380	107.7928	28.72184	158.1420	38.127	35.37	14.411	RSQ	P
Abductive (Graybill)	139.3475	107.7928	76.2382	63.1093	15.2153	14.12	6.212	0.551	0.0000
Sophistic	140.57180	97.0364	53.5011	87.0707	20.992	21.63	7.934		

This is expected since the test samples were near the center of the standard curve where the confidence interval is smallest

95	% C.I.	p=	0.05000						
	Upper CI	Mean	Lower CI	Range	s_{CA}	CV	SEM=		
Counter-Intuitive	?	?	?	?	?	?	?		
Intuitive	186.86380	107.7928	28.72184	158.1420	38.127	35.37	14.411	RSQ	P
Abductive (Graybill)	139.3475	107.7928	76.2382	63.1093	15.2153	14.12	6.212	0.551	0.0000
Sophistic	140.57180	97.0364	53.5011	87.0707	20.992	21.63	7.934		

The SEM for the abductive method is also much smaller and more accurately represents reality

From The Directions Tab:

ENTER THE VALUES OF YOUR STANDARD CURVE IN THE 'INPUTS & SUMMARIES' SPREADSHEET GREEN CELLS

IF YOU WOULD LIKE TO SEE YOUR CALIBRATION CURVE WITH 95% CONFIDENCE LIMITS ON THE INPUTS & SU... SHEET, THE AXES IN THE FIGURE AND DATA RANGE ... D TO BE ADJUSTED TO YOUR DATA POINTS

... THE 'INPUTS & SUMMARY' SPREADSHEET'S ...W CELLS

... INTERVAL IN THE 'INPUTS & SUMMARY' ...EET'S RED CELL

...PUTS SPREADSHEETS ...ND THE 'INPUTS & SUMMARY' SPREADSHEET

Always check to see if the standard <u>curve</u> is really a <u>curve</u>

IF YOUR DATA IS NOT LINEAR, A TRANSFORMATION MAY HELP. ADDITIONAL INSTRUCTIONS ARE INCLUDED ON THE "TRANSFORMATIONS" SPREADSHEET.

Is The Calibration Curve Really A Curve?

		Linear	Log10(x)	LN(x)	Sq Rt(x)	1 / x =	x^2	$1/x^2$	Sq Rt(x+.5)	Ln(x+1)	Cube Rt (x)	ln[x/(1-x)]	0.5ln [(1+x)/(1-x)]
	Factor...											1000	1000
	Inverse Transformation	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656
	m	-272.9300	-0.4559	-1.0496	-12.5333	0.1094	-46862.8451	0.0041	-12.4169	-0.9422	-2.8476	-8.2561	-0.2751
	b	0.8466	0.0055	0.0127	0.0509	-0.0002	130.9265	0.0000	0.0507	0.0125	0.0169	0.0137	0.0009
	R^2	**0.5506**	**0.6449**	**0.6449**	**0.5995**	**0.7081**	**0.4589**	**0.7280**	**0.5991**	**0.6434**	**0.6153**	**0.6393**	**0.5496**
Obs.	y_i	x_i											
1	357.798	31.675	1.501	3.456	5.628	0.032	1003.310	0.001	5.672	3.487	3.164	-3.420	0.032
2		31.675	1.501	3.456	5.628	0.032	1003.310	0.001	5.672	3.487	3.164	-3.420	0.032
		31.675	1.501										0.032
		31.675	1.501										0.032
			1.708										0.051
			1.708										0.051
			1.708										0.051
			1.708										0.051
			1.869										0.074
			1.869										0.074
			1.869										0.074
			1.869										0.074
			1.985										0.097
			1.985										0.097
			1.985										0.097
			1.985										0.097
		113.933	2.057										0.114
		113.933	2.057										0.114
		113.933	2.057										0.114
		113.933	2.057										0.114
21	457.198	135.931	2.133										0.137
22	447.067	135.931	2.133										0.137
23	473.275	135.931	2.133										0.137
24	436.685	135.931	2.133										0.137
25													

Observe the
“Transformations”
spreadsheet

Simply observe the R^2 values to determine which of these transformations will be most helpful to improve the fit of your data to a straight line. Or try a different one that could more suitable for your data.

If a transformation column shows #NUM for every value, there could be a problem with the formula dividing by zero. To avoid this problem, simply change the factor in Row 2 to 10, then 100, then 1000, etc. and stop when numbers first appear.

When you have chosen a transformation to use, paste the values for that column to Column B on the 'Inputs & Summary' spreadsheet. The results presented will be for the transformed data and need to be inverse transformed to linear space to be interpreted.

The formulas to inverse transform the results appear in Row 2 above. The values in Row 3 and Row 8 should be the same. Rows 8 to 54 transform the data and Row 3 has an example to display the inverse transformation code. The results on the Inputs & Summary spreadsheet will need to be inverse transformed manually

		Linear	Log10(x)	LN(x)	Sq Rt(x)	1 / x =	x^2	$1/x^2$	Sq Rt(x+.5)	Ln(x+1)	Cube Rt (x)	ln[x/(1-x)]	0.5ln [(1+x)/(1-x)]
	Factor...											1000	1000
	Inverse Transformation	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656
	m	-272.9300	-0.4559	-1.0496	-12.5333	0.1094	-46862.8451	0.0041	-12.4169	-0.9422	-2.8476	-8.2561	-0.2751
	b	0.8466	0.0055	0.0127	0.0509	-0.0002	130.9265	0.0000	0.0507	0.0125	0.0169	0.0137	0.0009
	R^2	**0.5506**	**0.6449**	**0.6449**	**0.5995**	**0.7081**	**0.4589**	**0.7280**	**0.5991**	**0.6434**	**0.6153**	**0.6393**	**0.5496**
Obs.	y_i	x_i											
1	[illegible]	31.675	1.501	3.456	5.628	0.032	1003.310	0.001	5.672	3.487	3.164	-3.420	0.032
2	344.360	[illegible]	1.501	3.456	5.628	0.032	1003.310	0.001	5.672	3.487	3.164	-3.420	0.032
3	376.245	[illegible]	1.501										0.032
4	378.016	[illegible]	1.501										0.032
5	383.679	[illegible]	1.708										0.051
6	427.800	[illegible]	1.708										0.051
7	427.094	51.058	1.708										
8	435.683	51.058	1.708										
9	433.117	73.906	1.869										
10	425.500	73.906	1.869										
11	417.771	73.906	1.869										
12	441.892	73.906	1.869										
13	418.666	96.649	1.985										
14	434.649	96.649	1.985										
15	451.188	96.649	1.985										
16	446.331	96.649	1.985										
17	420.226	113.933	2.057										
18	432.314	113.933	2.057										
19	404.861	113.933	2.057										[illegible]
20	442.608	113.933	2.057										0.114
21	457.198	135.931	2.133										0.137
22	447.067	135.931	2.133										0.137
23	473.275	135.931	2.133										0.137
24	436.685	135.931	2.133										0.137
25													

Observe the r^2 values in Row 6 to determine which of these transformations will be most helpful to improve the fit of your data to a straight line. Or try a different transformation that could be more suitable for your data.

		Linear	Log10(x)	LN(x)	Sq Rt(x)	1/x =	x^2	$1/x^2$	Sq Rt(x+.5)	Ln(x+1)	Cube Rt (x)	ln[x/(1-x)]	0.5ln [(1+x)/(1-x)]
	Factor...											1000	1000
	Inverse Transformation	31.6750656	31.6750656	31.6750656	31.6750656	31.675065	50656	31.6750656	31.67506	656	31.6750656	31.6750656	31.6750656
	m	-272.9300	-0.4559	-1.0496	-12.5333	0.1094	-4	0.0041	-12.4169	-0.	-2.8476	-8.2561	-0.2751
	b	0.8466	0.0055	0.0127	0.0509	-0.0002	130.	0.0000	0.0507	0.0125	0.0169	0.0137	0.0009
	R^2	**0.5506**	**0.6449**	**0.6449**	**0.5995**	**0.7081**	**0.4589**	**0.7280**	**0.5991**	**0.6434**	**0.6153**	**0.6393**	**0.5496**
Obs.	y_i	x_i											
1	357.798	31.675	1.501	3.456	5.628	0.032	1003.310		5.672			-3.420	0.032
2	344.360	31.675	1.501	3.456	5.628		310					-3.420	0.032
3	376.245	31.675	1.501										0.032
4	378.016	31.675											0.032
5	383.679	51.0											0.051
6	427.800	5											0.051
7	427.094												51
8	435.683												1
9	433.117												74
10	425.500												74
11	4												4
12													
13													
14													
15													
16	4												
17	420.2												4
18	43												0.114
19	4												0.114
20	4												0.114
21	45												0.137
22	447.0												0.137
23	473.275												0.137
24	436.685	135.931											0.137
25													

When A transformation has been chosen, paste the values from that column to Column B on the 'inputs & summary' spreadsheet. The results presented will be for the transformed data and need to be inverse transformed to linear space to be interpreted.

The formulas to inverse transform the results appear in Row 3. The values in Row 3 and Cell C8 should be the same. Rows 8 to 54 transform the data. The results on the Inputs & Summary spreadsheet will need to be inverse transformed manually

		Linear	Log10(x)	LN(x)	Sq Rt(x)	1 / x =	x^2	$1/x^2$	Sq Rt(x+.5)	Ln(x+1)	Cube Rt (x)	ln[x/(1-x)]	0.5ln [(1+x)/(1-x)]
	Factor...											1000	1000
	Inverse Transformation	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	[illegible]	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656	31.6750656
	m	-272.9300	-0.4559	-1.0496	-12.5333	0.1094	-46862.8451	0.0041	-12.4169	-0.9422	-2.8476	-8.2561	-0.2751
	b	0.8466	0.0055	0.0127	0.0509	-0.0002	130.9265	0.0000	0.0507	0.0125	0.0169	0.0137	0.0009
	R^2	**0.5506**	**0.6449**	**0.6449**	**0.5995**	**0.7081**	**0.4589**	**0.7280**	**0.5991**	**0.6434**	**0.6153**	**0.6393**	**0.5496**
Obs.	y_i	x_i											
1	357.798	31.675	[illegible]	3.456	5.628	0.032	1003.310	0.001	[illegible]	3.487	3.164	-3.420	0.032
2	344.360	31.675	1.501	3.456	5.628	0.032	1003.310	0.001	5.672	3.487	3.164	-3.420	0.032
3	376.245	31.675	1.501	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.032
4	378.016	31.675	1.501	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.032
5	383.679	51.058	1.708	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.051
6	427.800	51.058	1.708	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.051

Simply observe the R^2 values to determine which of these transformations will be most helpful to improve the fit of your data to a straight line. Or try a different one that could more suitable for

In this example the data were log_{10} transformed, the line was fitted and the ci was calculated. The results then had to be inverse transformed (raised to the x power) to be interpreted in normal space. Note that the mean is no longer in the center of the CI.

	95 % C.I.	p= 0.05			
	Upper CI	Mean	Lower CI	Range	R^2
Linear Standard Curve					
Intuitive	127.4	106.9	86.3	41.1	0.640
Abductive - Graybill	130.2	106.9	83.5	46.6	0.640
Transformed Standard Curve : $log_{10}(x)$					
Intuitive	2.1248132	2.0070633	1.8893133	0.2	0.712
Abductive - Graybill	2.1261638	2.0070633	1.8879627	0.2	0.712
Inverse Transformed : 10^x					
Intuitive	133.3	101.6	77.5	55.8	
Abductive - Graybill	133.7	101.6	77.3	56.4	

95 % C.I.		p= 0.05			
	Upper CI	Mean	Lower CI	Range	R^2
Linear Standard Curve					
Intuitive	127.4	106.9	86.3	41.1	0.640
Abductive - Graybill	130.2	106.9	83.5	46.6	0.640
Transformed Standard Curve : $\log_{10}(x)$					
Intuitive	2.1248132	2.0070633	1.8893133	0.2	0.712
Abductive - Graybill	2.1261638	2.0070633	1.8879627	0.2	0.712
Inverse Transformed : 10^x					
Intuitive	133.3	101.6	77.5	55.8	
Abductive - Graybill	133.7	101.6	77.3	56.4	

In this example the range is larger for the abductive than the intuitive methods. That is because the test sample values were near the extremes of the standard curve where the confidence interval is wider.

R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG

ENTER YOUR CALIBRATION CURVE DATA INTO THE "INPUTS & OUTPUTS" SPREADSHEET

ENTER YOUR "UNKNOWN" RESULTS STARTING IN COLUMN R, CELLS 5 TO 14.

THE RESULTS OF ABDUCTIVE, INTUITIVE AND SOPHISTIC MODELS ARE DISPLAYED BELOW THE INPUTS.

YOU CAN COPY COLUMN AZ TO AS MANY COLUMNS AS YOU'D LIKE

REPLICATE NUMBER	SAMPLE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	419.80													
2	391.44													
3	462.76													
4	457.14													
5	452.70													
6	438.05													
7	436.98													
8														
9														
10														
n_u	7	0	0											
ABDUCTIVE METHOD														
Predicted Test Sample Mean	107.79	#DIV/0!												
SD of Test Sample	15.22	#DIV/0!												
CV	14.12	#DIV/0!												
SEM of Test Sample	6.21	#DIV/0!												
INTUITIVE METHOD														
Predicted Test Sample Mean	107.79	#DIV/0!	#DIV											
SD of Test Sample	38.13	#DIV/0!	#DIV/											
CV	35.37	#DIV/0!	#D											
SEM of Test Sample	15.57	#DIV/0!	#I											
SOPHISTIC METHOD														
Predicted Test Sample Mean	97.04	#D	#D											
SD of Test Sample	20.99	#DIV/	DIV/											
CV	21.63	#DIV/0!	/0!											
SEM of Test Sample	8.57	#DIV/0!		#DIV/0.										

Abductive (Graybill) Model | Intuitive Method | Sophistic Method | Lab Spreadsheet | Transformations | EV2 Model | Simulations | Slope Comparison Chart

For multiple test samples, choose the "Lab Spreadsheet" tab

Enter test sample replicates (up to 10) into columns "T" and above. Observe the results at the bottom of each column

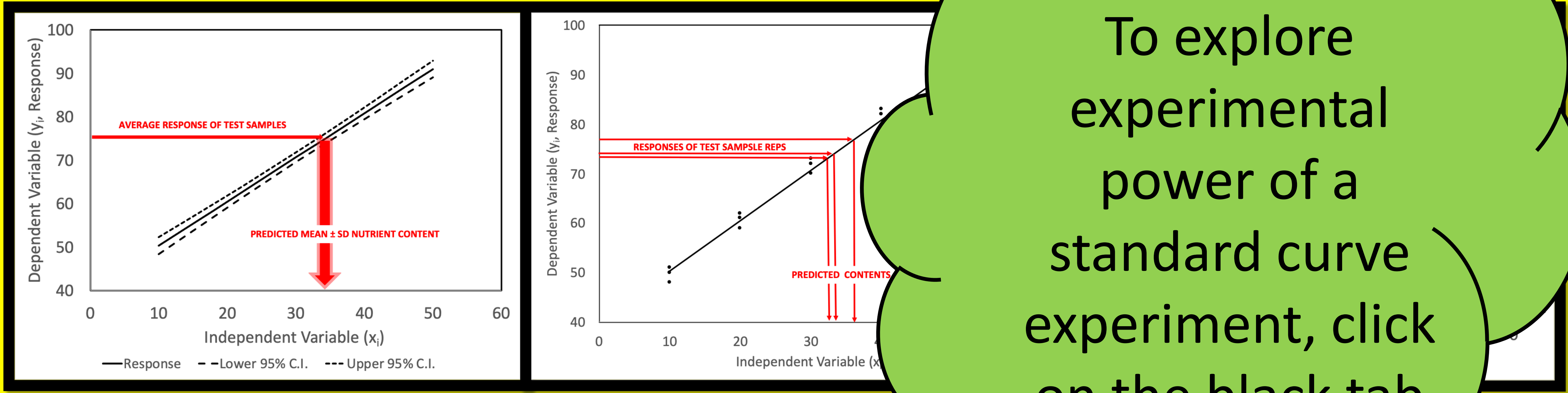
Graybill's Abductive Method Should Only Improve Your Results

It can also be helpful in
estimating experimental power.

CALIBRATION CURVE CONFIDENCE CALCULATOR

TEST SAMPLE VALUES SHOULD BE EVALUATED LIKE THIS

NOT LIKE THIS

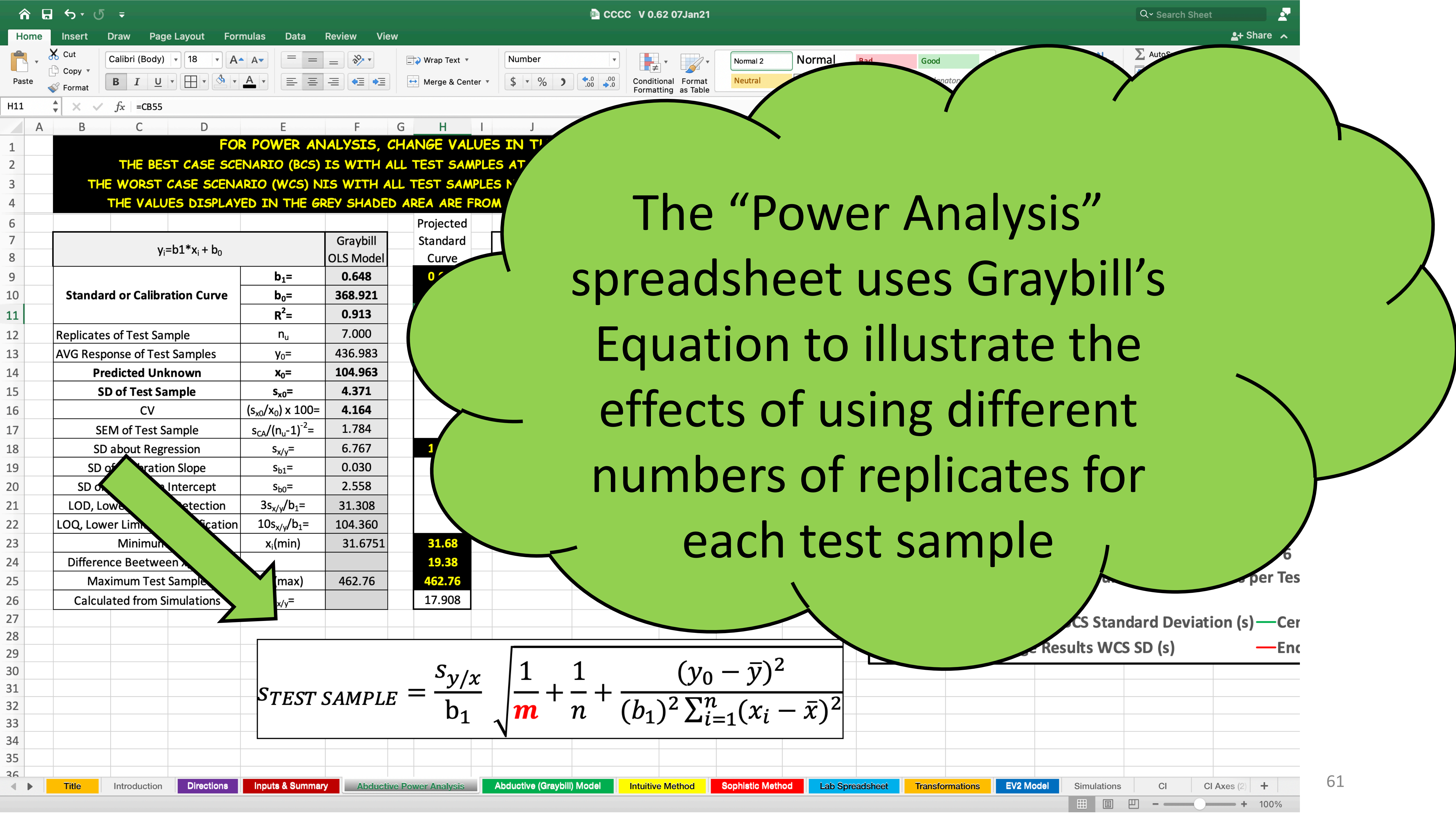


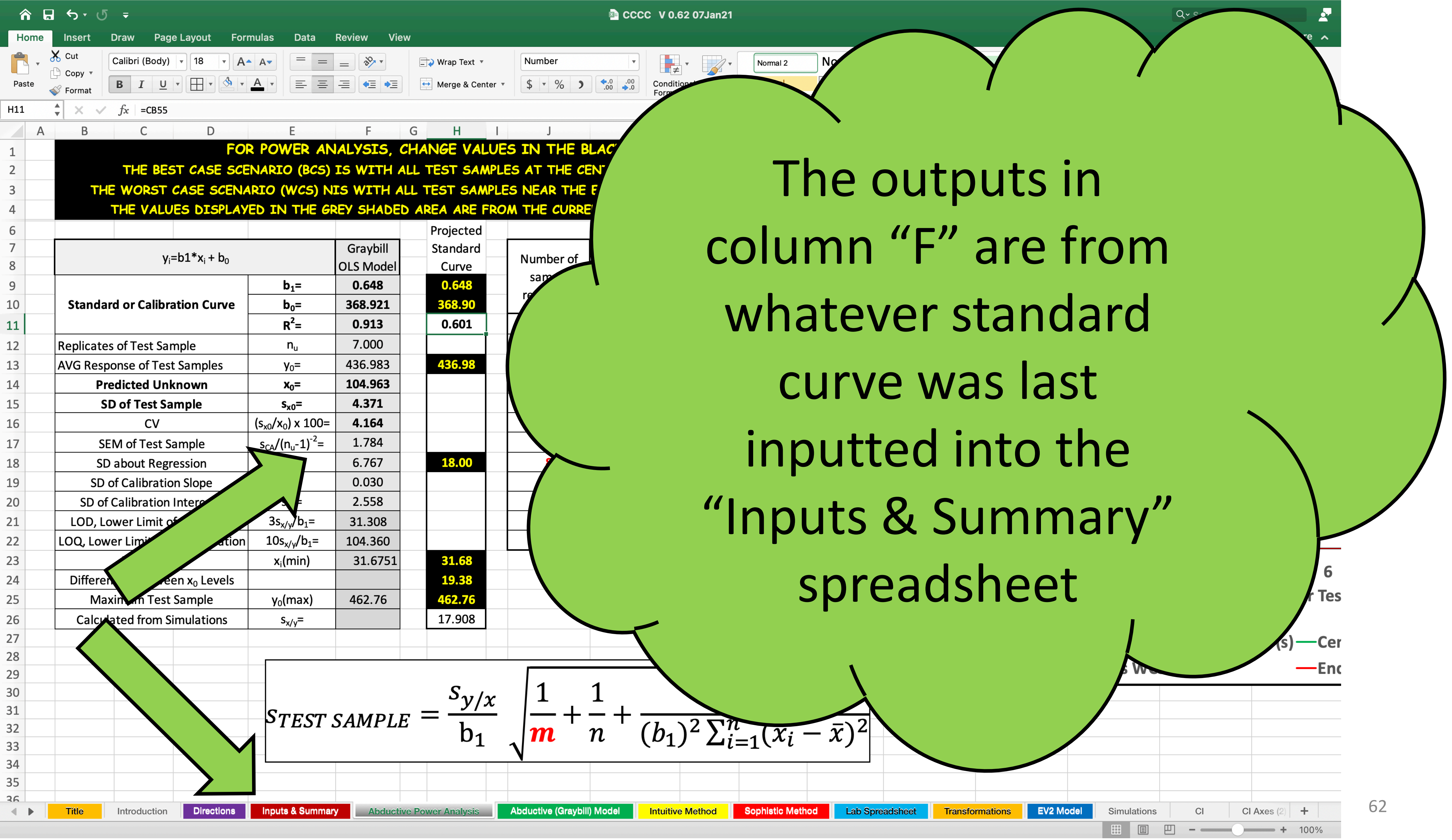
To explore experimental power of a standard curve experiment, click on the black tab

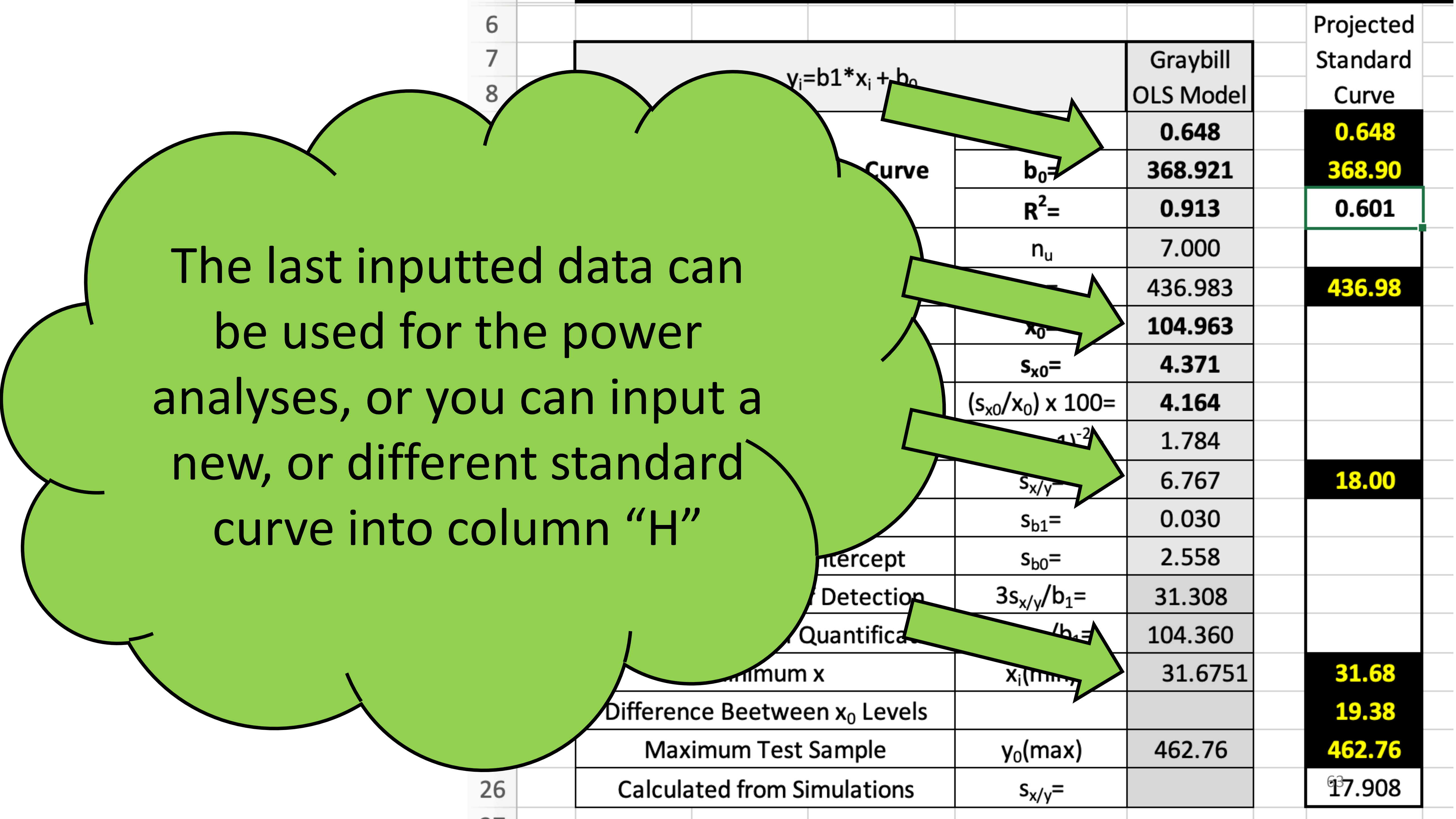
GM Pesti
Greensboro, GA USA

Version 0.62

Title | Introduction | Directions | Inputs & Summary | Abductive Power Analysis | Abductive (Graybill) Model | Intuitive Method | Sophistic Method | Lab Spreadsheet | Transformations | EV2 Model | Simulations | CI | CI Axes (2)

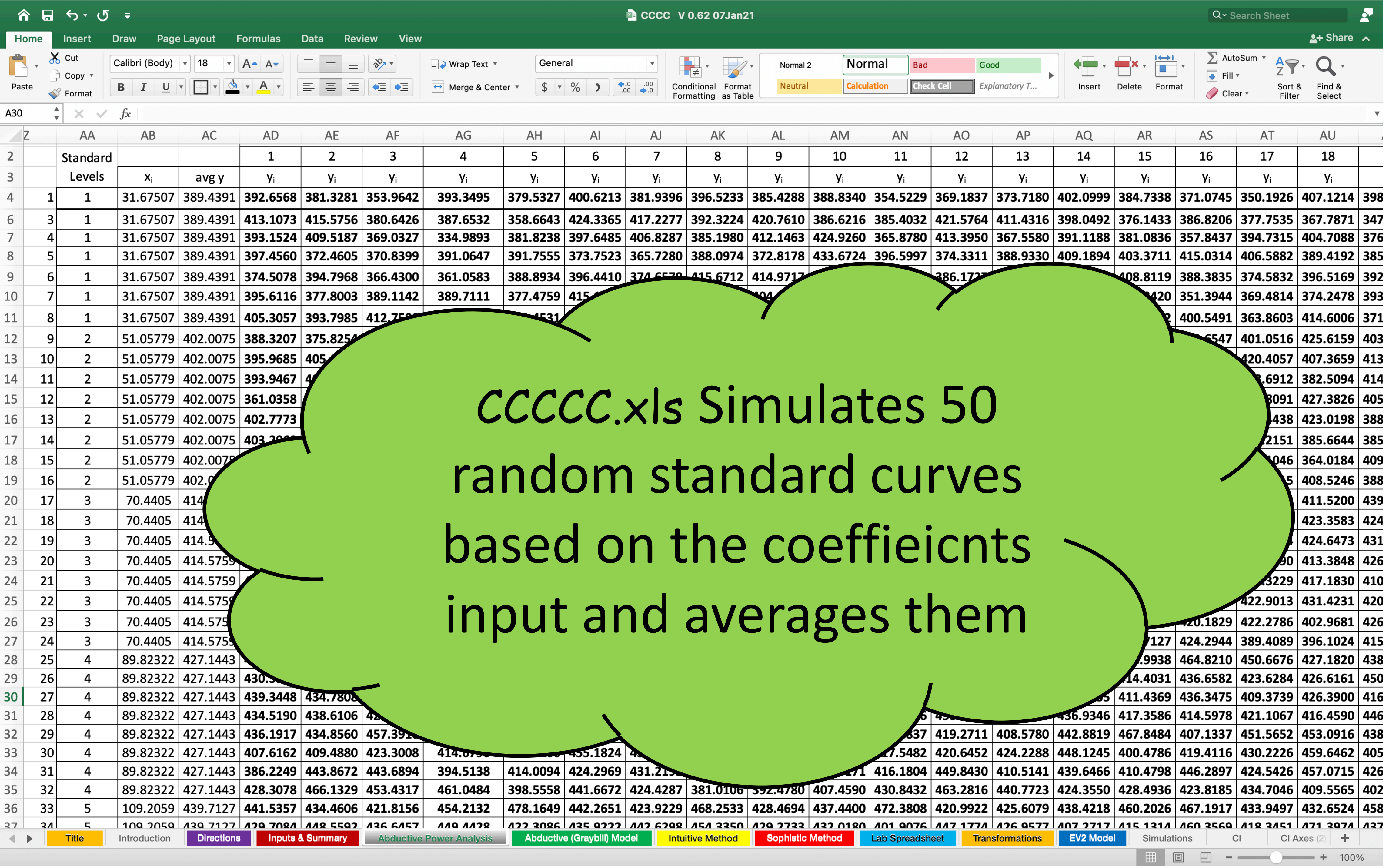

The "Power Analysis" spreadsheet uses Graybill's Equation to illustrate the effects of using different numbers of replicates for each test sample
CCCC V 0.62 07Jan21
FOR POWER ANALYSIS, CHANGE VALUES IN T
THE BEST CASE SCENARIO (BCS) IS WITH ALL TEST SAMPLES AT
THE WORST CASE SCENARIO (WCS) NIS WITH ALL TEST SAMPLES N
THE VALUES DISPLAYED IN THE GREY SHADED AREA ARE FROM
$y_i=b1*x_i+b_0$
Graybill OLS Model
Projected Standard Curve
Standard or Calibration Curve
$b_1=$ 0.648
$b_0=$ 368.921
$R^2=$ 0.913
Replicates of Test Sample n_u 7.000
AVG Response of Test Samples $y_0=$ 436.983
Predicted Unknown $x_0=$ 104.963
SD of Test Sample $s_{x0}=$ 4.371
CV $(s_{x0}/x_0)\times 100=$ 4.164
SEM of Test Sample $s_{CA}/(n_u-1)^{-2}=$ 1.784
SD about Regression $s_{x/y}=$ 6.767
$s_{b1}=$ 0.030
$s_{b0}=$ 2.558
$3s_{x/y}/b_1=$ 31.308
$10s_{x/y}/b_1=$ 104.360
$x_i(\min)$ 31.6751 31.68
19.38
Maximum Test Sample 462.76 462.76
Calculated from Simulations 17.908
$$s_{TEST\ SAMPLE}=\frac{s_{y/x}}{b_1}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{(b_1)^2\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$
Title | Introduction | Directions | Inputs & Summary | Abductive Power Analysis | Abductive (Graybill) Model | Intuitive Method | Sophistic Method | Lab Spreadsheet | Transformations | EV2 Model | Simulations | CI | CI Axes (2)


The outputs in column “F” are from whatever standard curve was last inputted into the “Inputs & Summary” spreadsheet
FOR POWER ANALYSIS, CHANGE VALUES IN THE BLAC
THE BEST CASE SCENARIO (BCS) IS WITH ALL TEST SAMPLES AT THE CEN
THE WORST CASE SCENARIO (WCS) NIS WITH ALL TEST SAMPLES NEAR THE E
THE VALUES DISPLAYED IN THE GREY SHADED AREA ARE FROM THE CURRE
Graybill OLS Model
Projected Standard Curve
Standard or Calibration Curve
Replicates of Test Sample
AVG Response of Test Samples
Predicted Unknown
SD of Test Sample
CV
SEM of Test Sample
SD about Regression
SD of Calibration Slope
Inputs & Summary
Abductive Power Analysis
Abductive (Graybill) Model
Intuitive Method
Sophistic Method
Lab Spreadsheet
Transformations
EV2 Model
Simulations


The last inputted data can be used for the power analyses, or you can input a new, or different standard curve into column "H"
Graybill OLS Model
Projected Standard Curve
y_i=b1*x_i + b_0
Curve
b_0=
R^2=
n_u
x_0=
s_{x0}=
(s_{x0}/x_0) x 100=
$s_{x/y}$=
s_{b1}=
s_{b0}=
$3s_{x/y}/b_1$=
x_i(min)
0.648
368.921
0.913
7.000
436.983
104.963
4.371
4.164
1.784
6.767
0.030
2.558
31.308
104.360
31.6751
462.76
0.648
368.90
0.601
436.98
18.00
31.68
19.38
462.76
17.908
Difference Beetween x_0 Levels
Maximum Test Sample
y_0(max)
Calculated from Simulations
$s_{x/y}$=

y_i=b1*x_i + b_0		Graybill OLS Model	Projected Standard Curve
Standard or Calibration Curve	**b_1=**	**0.648**	**0.648**
	b_0=	**368.921**	**368.90**
	R^2=	**0.913**	**0.601**
Replicates of Test Sample	n_u	7.000	
AVG Response of Test Samples	y_0=	436.983	**436.98**
Predicted Unknown	**x_0=**	**104.963**	
SD of Test Sample	**s_{x0}=**	**4.371**	
CV	(s_{x0}/x_0) x 100=	**4.164**	
SEM of Test Sample	$s_{CA}/(n_u-1)^{-2}$=	1.784	
SD about Regression	$s_{x/y}$=	6.767	**18.00**
SD of Calibration Slope	s_{b1}=	0.030	
SD of Calibration Intercept	s_{b0}=	2.558	
LOD, Lower Limit of Detection	$3s_{x/y}/b_1$=	31.308	
LOQ, Lower Limit of Quantification	$10s_{x/y}/b_1$=	104.360	
Minimum x	x_i(min)	31.6751	**31.68**
Difference Beetween x_0 Levels			**19.38**
Maximum Test Sample	y_0(max)	462.76	**462.76**
Calculated from Simulations	$s_{x/y}$=		17.908

The needed information includes the standard curve itself, information about the x values and SD of the regression

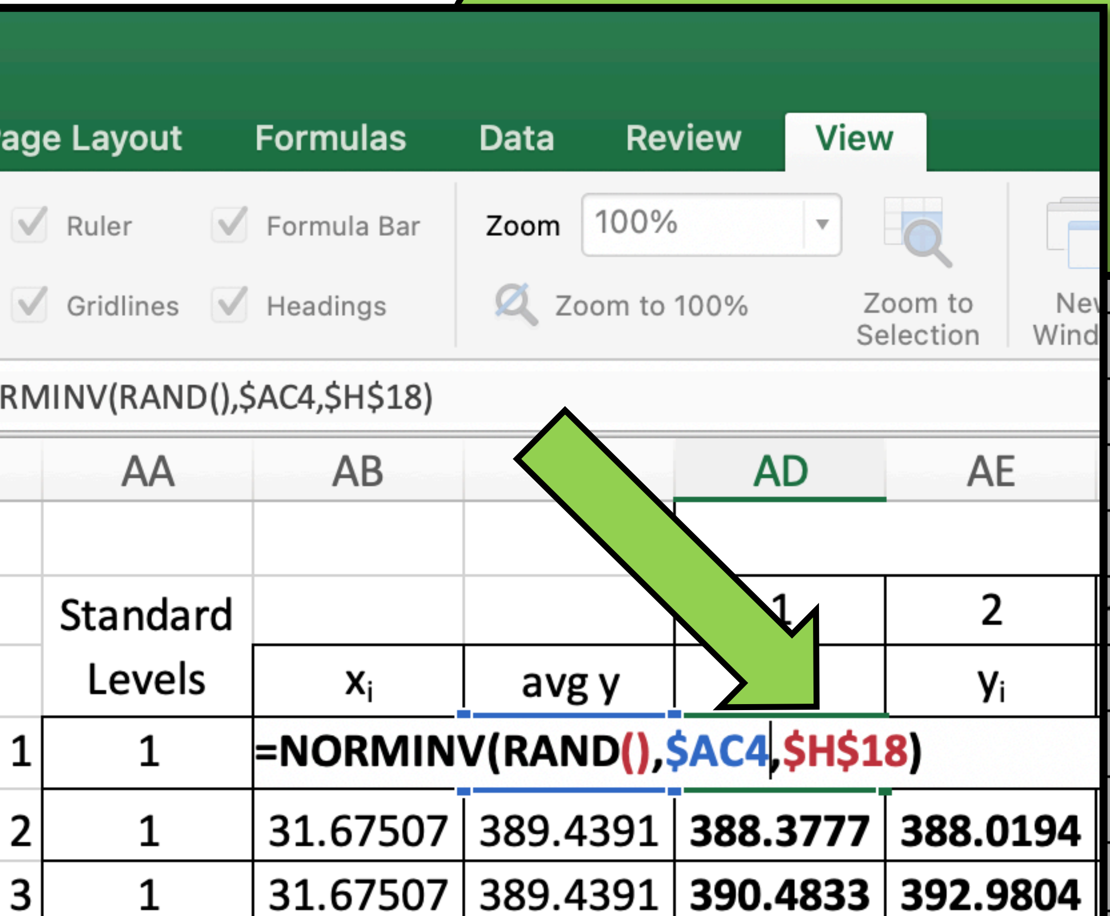


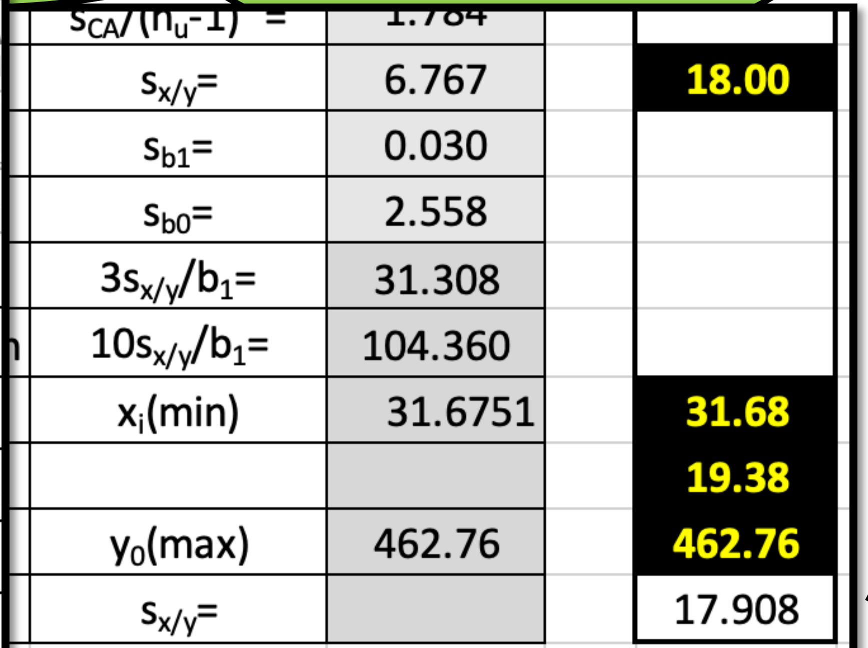
ccccc.xls Simulates 50 random standard curves based on the coeffieicnts input and averages them

The standard deviation of the regression is very close to the standard deviation of the standard's means in the standard curve.

SEM of Test Sample	$s_{CA}/(n_u-1)$ =	1.784		
SD about Regression	$s_{x/y}$=	6.767		**18.00**
SD of Calibration Slope	s_{b1}=	0.030		
SD of Calibration Intercept	s_{b0}=	2.558		
LOD, Lower Limit of Detection	$3s_{x/y}/b_1$=	31.308		
LOQ, Lower Limit of Quantification	$10s_{x/y}/b_1$=	104.360		
Minimum x	x_i(min)	31.6751		**31.68**
Difference Beetween x_0 Levels				**19.38**
Maximum Test Sample	y_0(max)	462.76		**462.76**
Calculated from Simulations	$s_{x/y}$=			17.908

The simulated standard curve is calculated from the standard deviations of the regressions. The values in ad4 to ca51 are from the same distribution using the same inputs





Number of sample replicates	Centered Results		Ends of Range Results	
	BCS Standard Deviation (s)	BCS Std. Error of Mean	WCS SD (s)	WCS SEM
1	28.21		29.12	
2	20.26	20.26	21.50	21.50
3	16.79	11.87	18.28	12.92
4	14.76	8.52	16.43	9.48
5	13.39	6.69	15.21	7.60
6	12.39	5.54	14.34	6.41
7	11.63	4.75	13.68	5.59
8	11.02	4.16	13.17	4.98
9	10.52	3.72	12.76	4.51
10	10.11	3.37	12.42	4.14
11	9.75	3.08	12.13	3.84
12	9.45	2.85	11.89	3.59

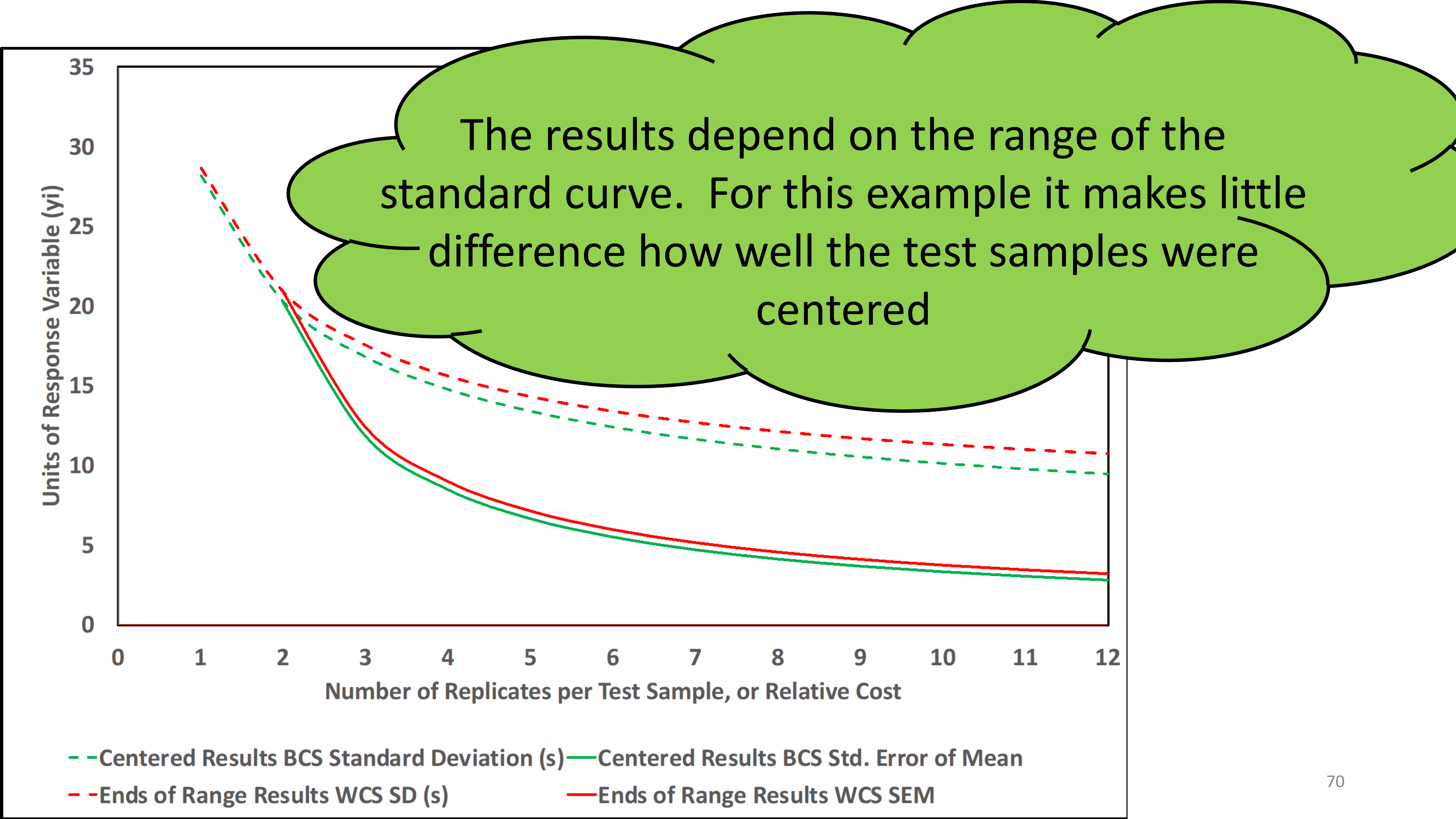
$$\sqrt{\frac{1}{\boldsymbol{m}}+\frac{1}{n}+\frac{(y_0-\bar{y})^2}{(b_1)^2\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$

The “M” term of Graybill’s Equation is then varied to estimate the SD & SEM of test samples in the center and extremes of the standard (or calibration) curve

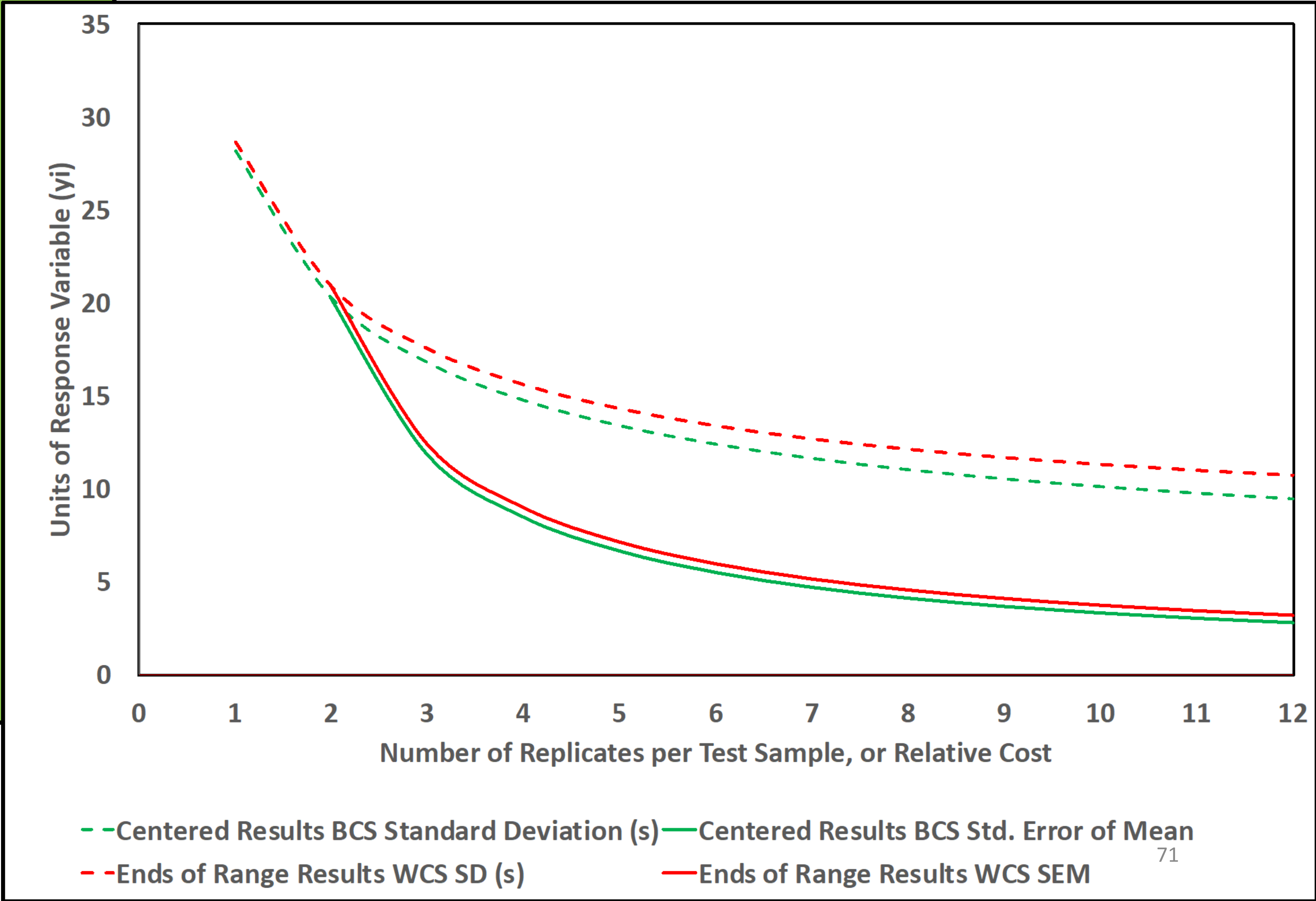
The SD & SEM of test samples in the center (Best-Case Scenario) and extremes (Worst-Case Scenario) of the calibration curve are calculated for different numbers of sample replicates, "M"

Number of sample replicates	Centered Results		Ends of Range Results	
	BCS Standard Deviation (s)	BCS Std. Error of Mean	WCS SD (s)	WCS SEM
1	28.21		29.12	
2	20.26	20.26	21.50	21.50
3	16.79	11.87	18.28	12.92
4	14.76	8.52	16.43	9.48
5	13.39	6.69	15.21	7.60
6	12.39	5.54	14.34	6.41
7	11.63	4.75	13.68	5.59
8	11.02	4.16	13.17	4.98
9	10.52	3.72	12.76	4.51
10	10.11	3.37	12.42	4.14
11	9.75	3.08	12.13	3.84
12	9.45	2.85	11.89	3.59

$$\sqrt{\frac{1}{\boldsymbol{m}} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{(b_1)^2 \sum_{i=1}^{n}(x_i - \bar{x})^2}}$$


The results depend on the range of the standard curve. For this example it makes little difference how well the test samples were centered
35
30
25
20
15
10
5
0
Units of Response Variable (yi)
0
1
2
3
4
5
6
7
8
9
10
11
12
Number of Replicates per Test Sample, or Relative Cost
Centered Results BCS Standard Deviation (s)
Centered Results BCS Std. Error of Mean
Ends of Range Results WCS SD (s)
Ends of Range Results WCS SEM

The interpretation of power curves may involve economic decisions. How much to pay for larger confidence in results?



Including Costs In Power Analyses

ENTER THE VALUES OF YOUR STANDARD CURVE IN THE GREEN CELLS

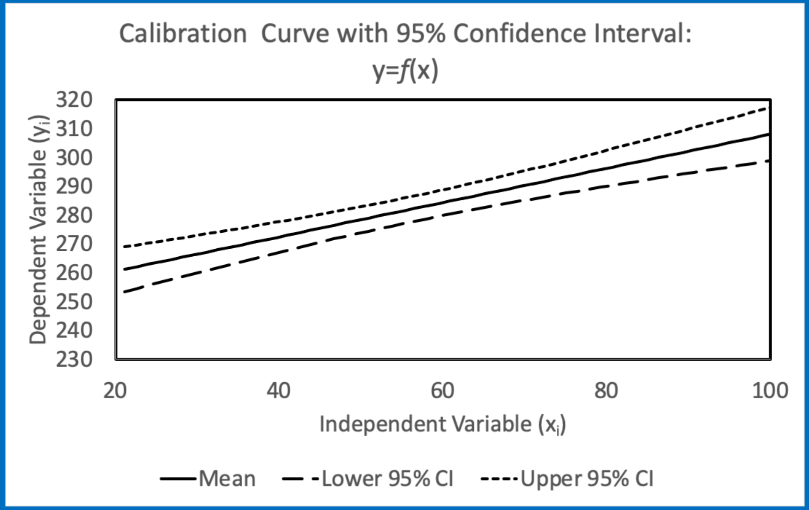
ENTER THE VALUES OF YOUR TEST SAMPLES IN THE YELLOW CELLS

YOUR VALUES WILL BE AUTOMATICALLY TRANSFERRED TO THE OUTPUT SPREADSHEETS

ENTER THE DESIRED CONFIDENCE INTERVAL FOR THE UNKNOWNS IN THE RED CELL

	Calibration Data		Test Sampe Responses
	C_{std}	S_{std}	
Obs.	x_i	y_i	
1	21	239	280
2	21	230	261
3	21	251	309
4	21	252	305
5	21	240	302
6	21	248	292
7	21	254	291
8	34	256	
9	34	285	
10	34	285	
11	34	290	
12	34	292	
13	34	292	
14	34	296	
15	49	289	
16	49	284	
17	49	279	
18	49	295	
19	49	286	
20	49	267	
21	49	286	
22	64	287	
23	64	279	
24	64	290	
25	64	301	
26	64	298	
27	64	285	
28	64	292	

95	% C.I.	p=	0.05000						
	Upper CL	Mean	Lower CL	Range	s_{CA}	CV	SEM=		
Counter-Intuitive	?	?	?	?	?	?	?		
Intuitive	127.7828	71.6456	15.5085	112.2744	27.776	38.77	10.498	RSQ	P
Abductive (Graybill)	91.8041	71.6456	51.4872	40.3169	9.9741	13.92	4.072	0.515	0.0000
Sophistic	92.9364	64.0148	35.0933	57.8431	14.310	22.35	5.409		



The Axes in the Figure, Must Be Re-set Manually for Each New Calibration Curve

<-- The example data is storred in Columns AO to AV

Independent Variable Minimum = 21	Dependent Variable Minimum = 230
Independent Variable Maximum = 91	Dependent Variable Maximum = 316

Title | Introduction | Directions | Inputs & Summary | SAVE OUTPUTS | Intuitive Method | Abductive (Graybill) Method | Sophistic Method | Abductive Power Analysis | Abductive Power Analysis (2) | Abductive Power (Costs) | Lab Spread

FOR POWER COST ANALYSIS, CHANGE VALUES IN THE GREEN CELLs ONLY. ALL OTHER VALUES ARE LINKED THROUGH THE "ABDUCTIVE POWER ANALYSIS (2) SPREADSHEET

Observed SD with	7	Obs. per Rep. =	21
Est. Variance of Reps.==>	1	Cost per Obs. ==>	$
Est. Variance of Obs. ==>	55.99	Cost Based per Rep ==>	$

y_i=b1*x_i + b_0		Graybill OLS Model	Projected Standard Curve	
Standard or Calibration Curve	b_1=	0.595	0.595	
	b_0=	373.026	373.026	
	R^2=	0.515	0.51[illegible]	
Replicates of Test Sample	m_u	7		
AVG Response of Test Samples	y_0=	436.983	[illegible]6.98	
Predicted Unknown	x_0=	107.468		
SD of Test Sample	s_{x0}=	14.961		
CV	(s_{x0}/x_0) x 100=	13.921		
SEM of Test Sample	$s_{CA}/(n_u-1)^{-2}$=	6.108		
SD about Regression	$s_{x/y}$=	21.161	33.324	
SD of Calibration Slope	s_{b1}=	0.091		
SD of Calibration Intercept	s_{b0}=	8.322		
LOD, Lower Limit of Detection	$3s_{x/y}/b_1$=	106.673		
LOQ, Lower Limit of Quantification	$10s_{x/y}/b_1$=	355.575		
Minimum x	x_i(min)	31.675	31.68	
Difference Beetween x_0 Levels			19.38	
Maximum Standard Response	y_0(max)	473.27	473.27	
Calculated from Simulations	$s_{x/y}$=		38.108	<--Compare to Cell H18
Calibration Curve Levels of x		6		
Calibration Curve Reps/Level of x		7		
Total n for Calibration Curve		42		

Number of sa[illegible]es	Centered Results		Ends of Range Results	
	BCS Standard Deviation (s)	BCS Std. Error of Mean	WCS SD (s)	WCS SEM
1	60.75		63.84	
2	43.15	43.15	47.40	47.40
3	35.38	25.02	40.46	28.61
4	30.77	17.77	36.50	21.07
5	27.64	13.82	33.90	16.95
6	25.34	11.33	32.05	14.33
7	23.56	9.62	30.66	12.52
8	22.13	8.36	29.58	11.18
9	20.95	7.41	28.70	10.15
10	19.96	6.65	27.99	9.33
11	19.11	6.04	27.39	8.66
12	18.37	5.54	26.88	8.10

[illegible]= Best-Case Scenario

[illegible]orst-Case Scenario

IN THIS MODEL m AND n ARE LINKED, n IS EQUAL TO m

Observations per Replicate	SD[illegible]	Total Costs per Relicate
		$
1	56.986	9.00
2	40.588	[illegible]13.00
3	33.324	[illegible].00
4	28.993	[illegible]0
5	26.038	[illegible]
6	23.856	29.00
7	22.161	33.00
8	20.794	37.00
9	19.662	41.00
10	18.704	45.00
11	17.881	49.00
12	17.162	53.00
13	16.528	57.00
14	15.963	61.00
15	15.456	65.00
16	14.997	69.00
17	14.579	73.00
18	14.196	77.00
19	13.844	81.00
		85.00
		89.00
		93.00
		97.00
		101.00
		105.00
		109.00
		113.00
		117.00
		121.00
		125.00
		129.00
		133.00
		137.00
		141.00

The variance is calculated from the SD about the regression and its sample size

The variance is calculated from the observed SD with its degrees of freedom.
Then the SD's for different numbers of observations per replicate are calculated from the variance

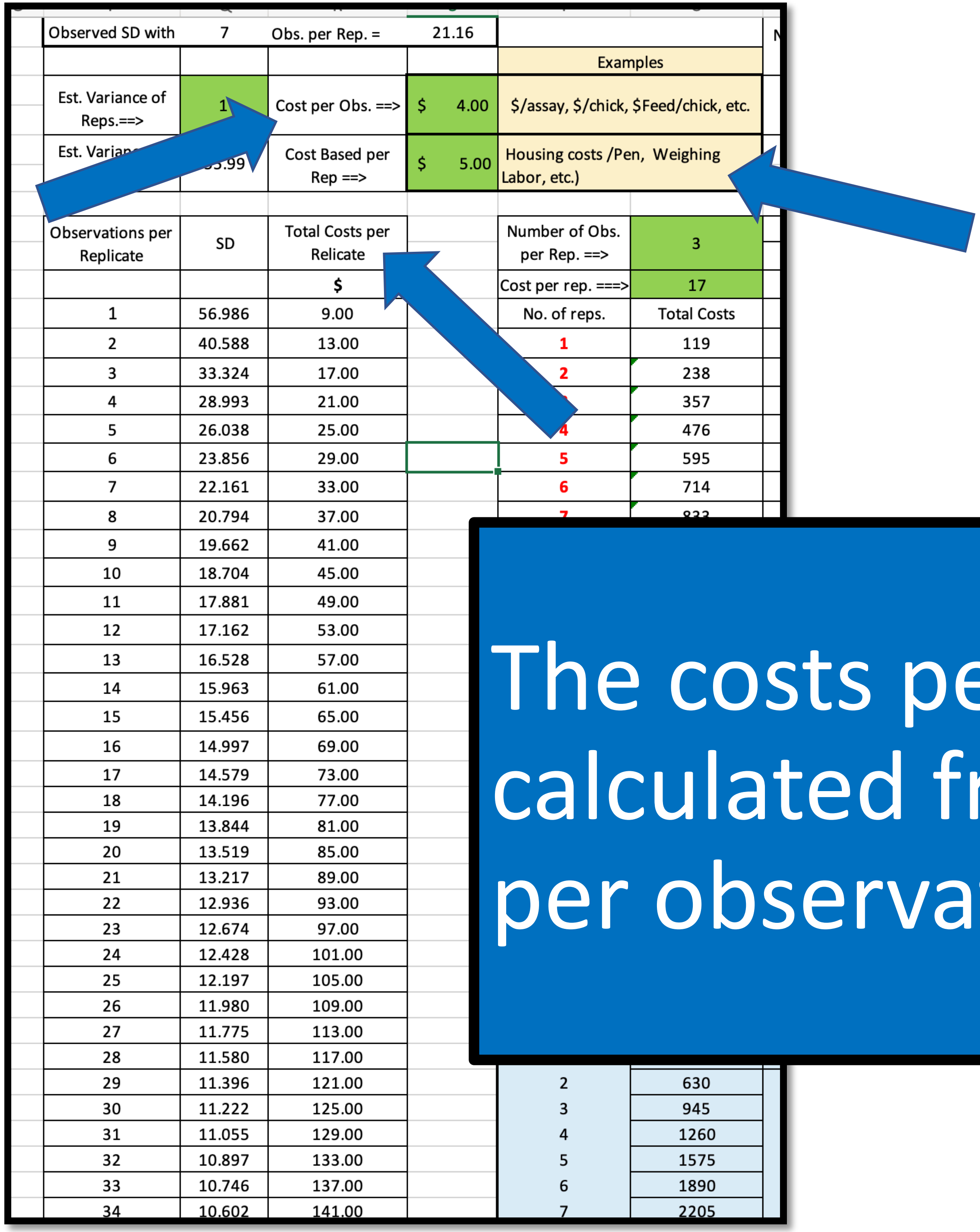
Observed SD with	7	Obs. per Rep. =	21.16
Est. Variance of Reps.==>	1	Cost per Obs. ==>	$ 4.00
Est. Variance of Obs. ==>	55.99	Cost Base... R...	$ 5.00

NKED

nds of Range Resu...

S SD	SEM
3.84	
7.40	47.40
0.46	28.61
6.50	21.07
3.90	16.95
2.05	14.33
0.66	12.52

Observations per Replicate	SD	...tal Costs per Relicate
		$
1	56.986	9.00
2	40.588	13.00
3	33.324	17.00
4	28.993	21.00
5	26.038	25.00
6	23.856	29.00
7	22.161	33.00
8	20.794	37.00
9	19.662	41.00
10	18.704	45.00
11	17.881	49.00
12	17.162	53.00
13	16.528	57.00
14	15.963	61.00
15	15.456	65.00
16	14.997	69.00
17	14.579	73.00
18	14.196	77.00
19	13.844	81.00
20	13.519	85.00
21	13.217	89.00
22	12.936	93.00
23	12.674	97.00
24	12.428	101.00
25	12.197	105.00
26	11.980	109.00
27	11.775	113.00
28	11.580	117.00
29	11.396	121.00
30	11.222	125.00
31	11.055	129.00
32	10.897	133.00
33	10.746	137.00
34	10.602	141.00



Observed SD with	7	Obs. per Rep. =	21.16		
				Examples	
Est. Variance of Reps.==>	1	Cost per Obs. ==>	$ 4.00	$/assay, $/chick, $Feed/chick, etc.	
Est. Varian	3.99	Cost Based per Rep ==>	$ 5.00	Housing costs /Pen, Weighing Labor, etc.)	
Observations per Replicate	SD	Total Costs per Relicate		Number of Obs. per Rep. ==>	3
		$		Cost per rep. ===>	17
1	56.986	9.00		No. of reps.	Total Costs
2	40.588	13.00		1	119
3	33.324	17.00		2	238
4	28.993	21.00		[illegible]	357
5	26.038	25.00		4	476
6	23.856	29.00		5	595
7	22.161	33.00		6	714
8	20.794	37.00		7	833
9	19.662	41.00			
10	18.704	45.00			
11	17.881	49.00			
12	17.162	53.00			
13	16.528	57.00			
14	15.963	61.00			
15	15.456	65.00			
16	14.997	69.00			
17	14.579	73.00			
18	14.196	77.00			
19	13.844	81.00			
20	13.519	85.00			
21	13.217	89.00			
22	12.936	93.00			
23	12.674	97.00			
24	12.428	101.00			
25	12.197	105.00			
26	11.980	109.00			
27	11.775	113.00			
28	11.580	117.00			
29	11.396	121.00		2	630
30	11.222	125.00		3	945
31	11.055	129.00		4	1260
32	10.897	133.00		5	1575
33	10.746	137.00		6	1890
34	10.602	141.00		7	2205

The costs per replicate are calculated from inputted costs per observation and replicate

GREEN CELLs ONLY. ALL OTHER VALUES ARE LINKED
'ER ANALYSIS (2) SPREADSHEET

Projected Standard Curve
0.595
373.026
0.515
436.98
33.324
31.68
19.38
473.27
38.108

<--Compare to Cell H18

Number of sample replicates	Centered Results: BCS Standard Deviation (s)	Centered Results: BCS Std. Error of Mean	Ends of Range Results: WCS SD (s)	Ends of Range Results: WCS SEM
1	60.75		63.84	
2	43.15	43.15	47.40	47.40
3	35.38	25.02	40.46	28.61
4	30.77	17.77	36.50	[illegible]
5	27.64	13.82	[illegible]	16.95
6	[illegible]	11.33	32.05	14.33
[illegible]	23.56	9.62	30.66	12.52
8	22.13	8.36	29.58	11.18
9	20.95	7.41	28.70	10.15
10	19.96	6.65	27.99	9.33
11	19.11	6.04	27.39	8.66
12	18.37			

BCS= Best-Case Scenario
WCS= Worst-Case Scenario

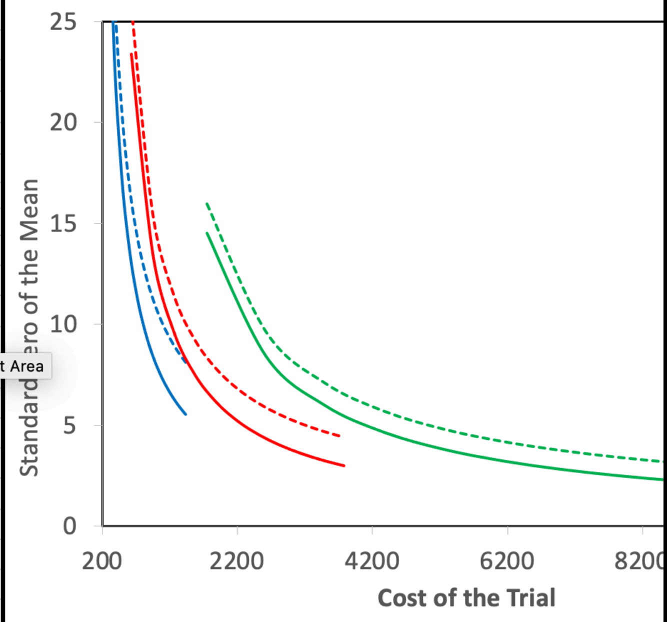
Observed SD with	7	Obs. per Rep. =	21.16		
				Examples	
Est. Variance of Reps.==>	1	Cost per Obs. ==>	$ 4.00	$/assay, $/chick, $Feed/chick, etc.	
Est. Variance of Obs. ==>	55.99	Cost Based per Rep ==>	$ 5.00	Housing costs /Pen, Weighing Labor, etc.)	

No. of Standard levels	6
No. of Test Samples	1
Observations per Rep. ==>	

Observations per Replicate	SD	Total Costs per Relicate
		$
1	56.986	9.00
2	[illegible]	[illegible]
3	33.324	17.00
4	[illegible]	[illegible]
5	26.038	25.00
6	23.856	29.00
7	22.161	33.00
8	20.794	37.00
9	19.662	41.00
10	18.704	45.00
11	17.881	49.00
32	[illegible]	[illegible]
33	10.746	137.00
34	10.602	141.00

Number of Obs. per Rep. ==>	3		
Cost per rep. ===>	17		
[illegible]	Total Costs	BCS SEM	WCS SEM
1	119		
2	238	43.15	47.40
3	357	25.02	28.61
4	476	17.77	21.07
5	595	13.82	16.95
6	714	11.33	14.33
7	833	9.62	12.52
8	952	8.36	11.18
9	1071	7.41	10.15
10	1190	6.65	9.33

5	[illegible]		[illegible]	[illegible]
6	1890		6.15	7.77
7	2205		5.22	6.79



SEM (30) | WCS SEM (30)

To calculate costs and estimate Standard Errors of the Mean of the test samples for any number of observations per replicate, copy the estimated SD and costs per replicate to cells H18 and U10.
Enter the number of observations per replicate in cell U9

L12 to L 22 and N 12 to N22 are transferred to cells V13 to W23 to facilitate copying for graphing

Number of sample replicates	Centered Results		Ends of Range Results	
	BCS Standard Deviation (s)	BCS Std. Error of Mean	WCS SD (s)	WCS SEM
1	60.75		63.84	
2	43.15	43.15	47.40	47.40
3	35.38	25.02	40.46	28.61
4	30.77	17.77	36.50	21.07
5	27.64	13.82	33.90	16.95
6	25.34	11.33	32.05	14.33
7	23.56	9.62	30.66	12.52
8	22.13	8.36	29.58	11.18
9	20.95	7.41	28.70	10.15
10	19.96	6.65	27.99	9.33
11	19.11	6.04	27.39	8.66
12	18.37	5.54	26.88	8.10

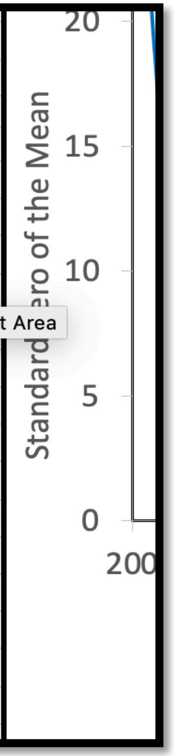
BCS= Best-Case Scenario

Est. Variance of Obs. ==>	55.99	Cost Based per Rep ==>	$ 5.00	Housing costs /Pen, Weighing Labor, etc.)

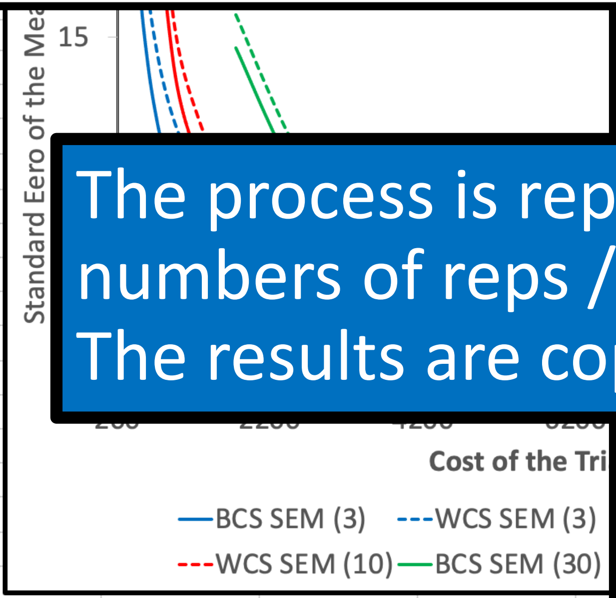
Observations per Replicate	SD	Total Costs per Relicate
		$
1	56.986	9.00
2	40.588	13.00
3	33.324	17.00
4	28.993	21.00
5	26.038	25.00
6	23.856	29.00
7	22.161	33.00
8	20.794	37.00
9	19.662	41.00
10	18.704	45.00
11	17.881	49.00
12	17.162	53.00
13	16.528	57.00

Observations per Rep. ==>

Number of Obs. per Rep. ==>	3		
Cost per rep. ===>	17		
No. of reps.	Total Costs	BCS SEM	WCS SEM
1	119		
2	238	43.15	47.40
3	357	25.02	28.61
4	476	17.77	21.07
5	595	13.82	16.95
6	714	11.33	14.33
7	833	9.62	12.52
8	952	8.36	11.18
9	1071	7.41	10.15
10	1190	6.65	9.33
11	1309	6.04	8.66
12	1428	5.54	8.10



Number of Obs. per Rep. ==>	3		
Cost per rep. ===>	17		
No. of reps.	Total Costs	BCS SEM	WCS SEM
1	119		
2	238	43.15	47.40
	357	25.02	28.61
	476	17.77	21.07
5	595	13.82	16.95
6	714	11.33	14.33
7	833	9.62	12.52
8	952	8.36	11.18
9	1071	7.41	10.15
10	1190	6.65	9.33
11	1309	6.04	8.66
12	1428	5.54	8.10



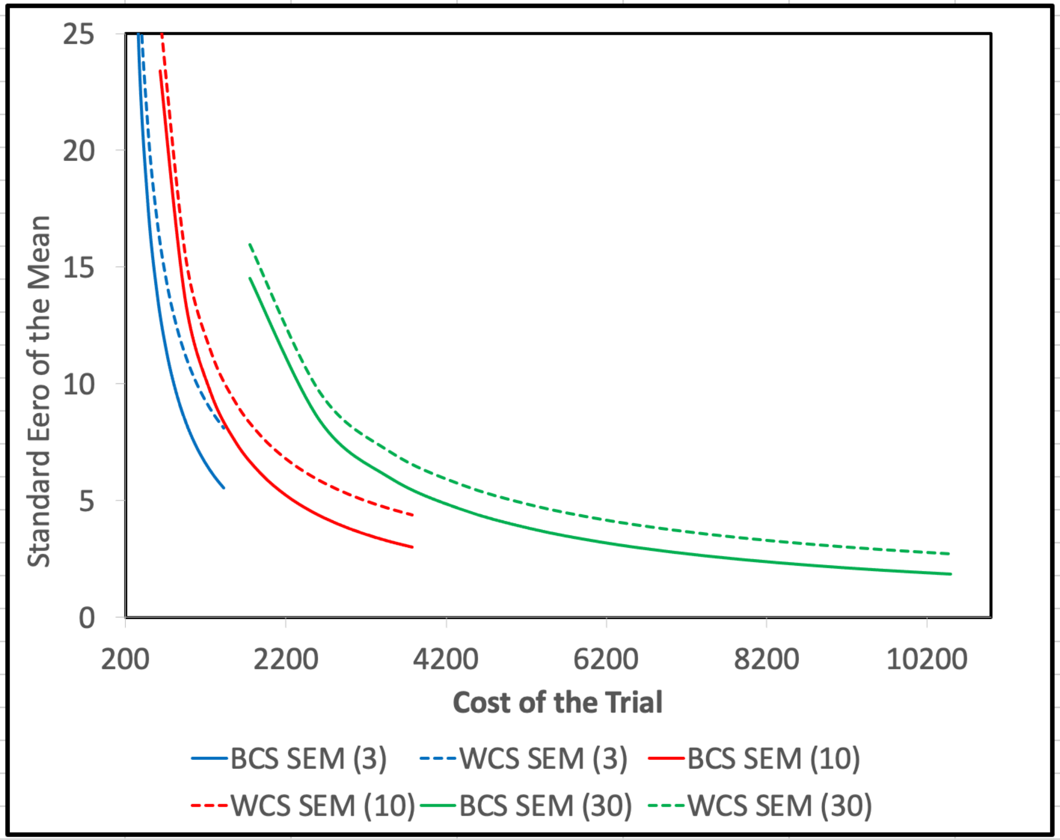
The process is repeated 3 times for different numbers of reps / treatment.
The results are copied to cells U28 to AA60.

DATA FOR GRAPHING

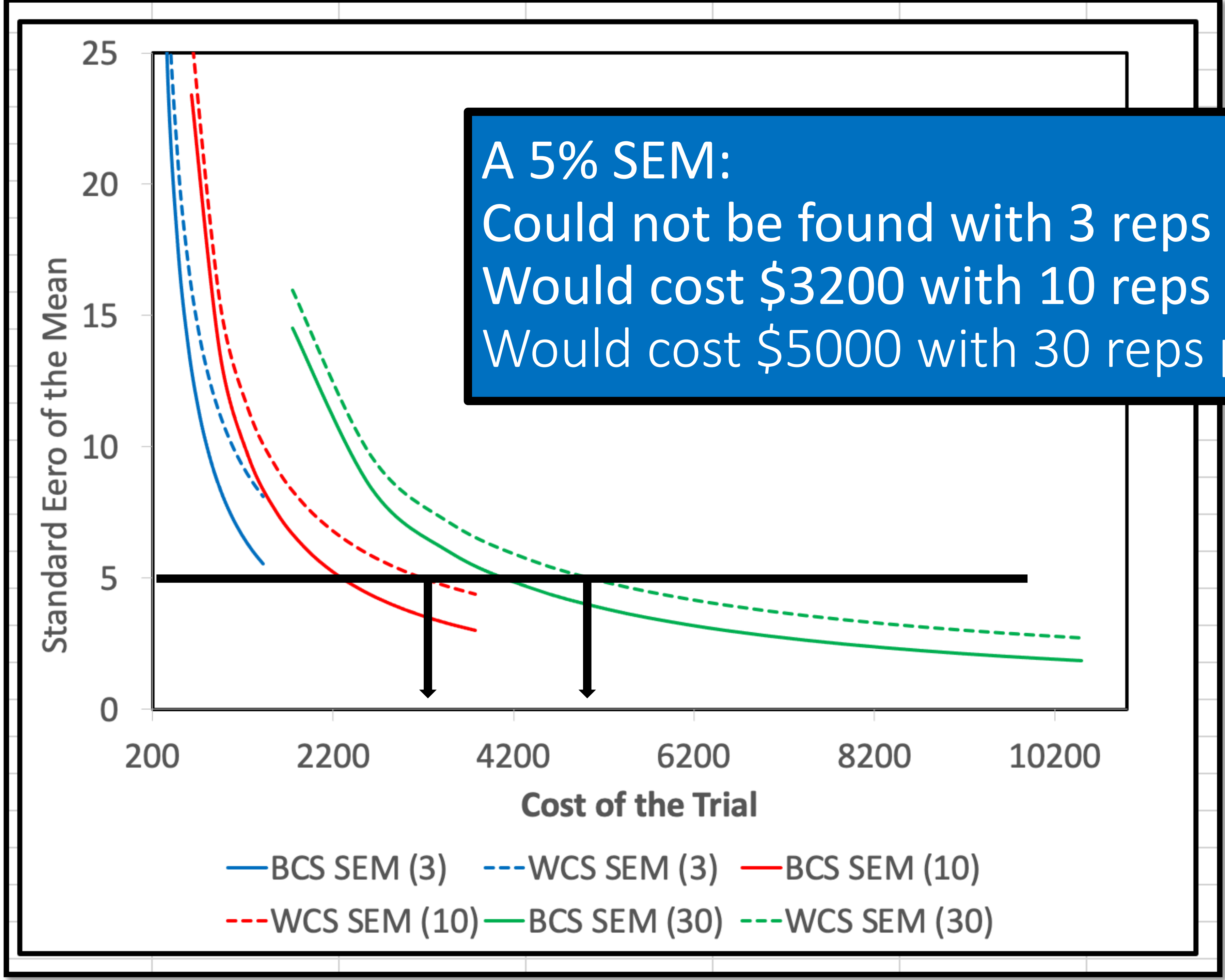
	Total Costs	BCS SEM (3)	WCS SEM (3)	BCS SEM (10)	WCS SEM (10)	BCS SEM (30)	WCS SEM (30)
2	238	43.15	47.40				
3	357	25.02	28.61				
4	476	17.77	21.07				
5	595	13.82	16.95				
6	714	11.33	14.33				
7	833	9.62	12.52				
8	952	8.36	11.18				
9	1071	7.41	10.15				
10	1190	6.65	9.33				
11	1309	6.04	8.66				
12	1428	5.54	8.10				
2	630			23.40	25.71		
3	945			13.57	15.52		
4	1260			9.64	11.43		
5	1575			7.50	9.19		
6	1890			6.15	7.77		
7	2205			5.22	6.79		
8	2520			4.54	6.06		
9	2835			4.02	5.50		
10	3150			3.61	5.06		
11	3465			3.28	4.70		

Summary | Intuitive Method | Abductive (Graybill) Method | Sophistic Method | Abductive Power Analysis | Abductive Powe

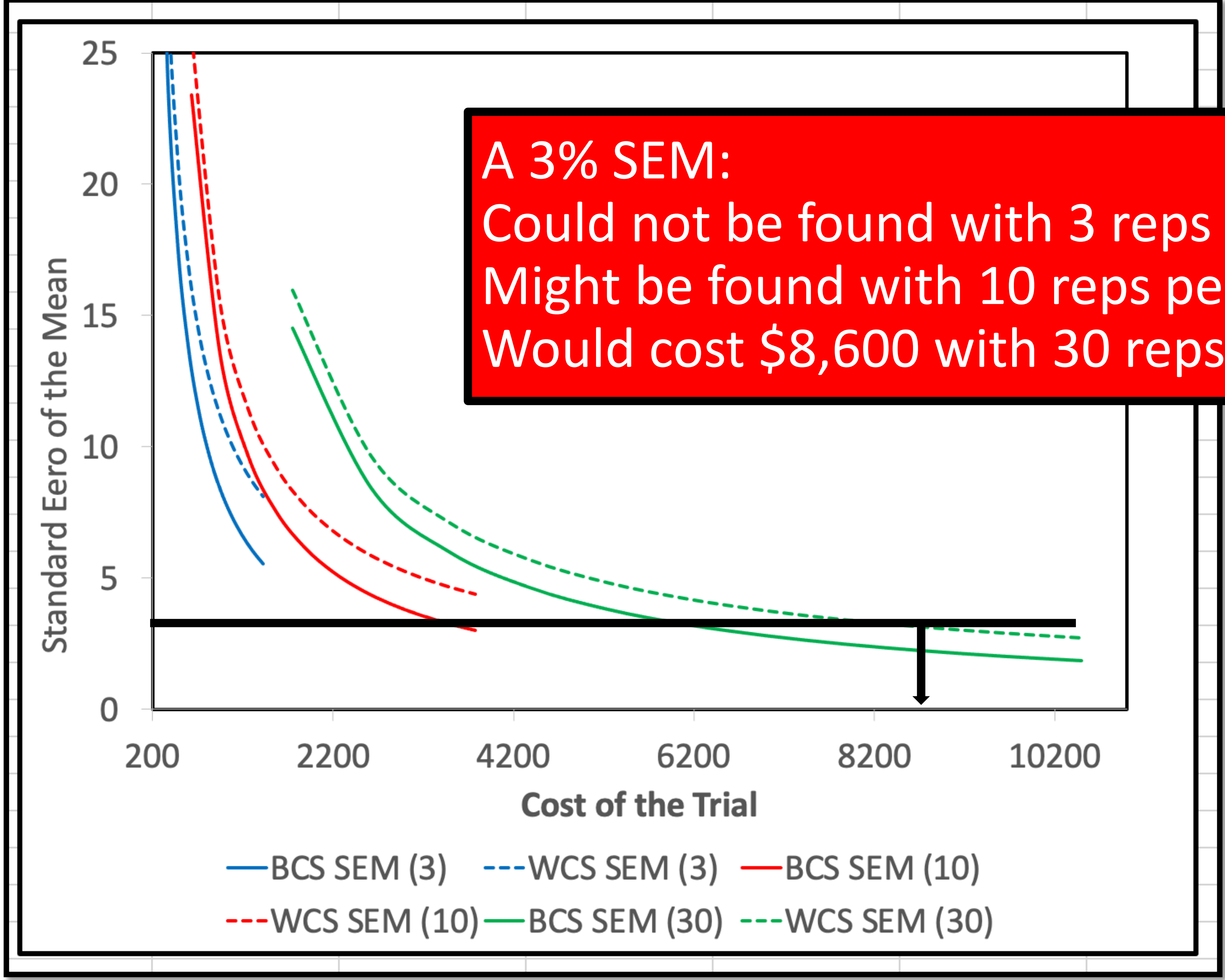
DATA FOR GRAPHING							
	Total Costs	BCS SEM (3)	WCS SEM (3)	BCS SEM (10)	WCS SEM (10)	BCS SEM (30)	WCS SEM (30)
2	238	43.15	47.40				
3	357	25.02	28.01				
4	476	17.77	21.07				
5	595	13.82	16.95				
6	714	11.33	14.33				
7	833	9.62	12.52				
8	952	8.36	11.18				
9	1071	7.41	10.15				
10	1190	6.65	9.33				
11	1309	6.04	8.66				
12	1428	5.54	8.10				
2	630			23.40	25.71		
3	945			13.57	15.52		
4	1260			9.64	11.43		
5	1575			7.50	9.19		
6	1890			6.15	7.77		
7	2205			5.22	6.79		
8	2520			4.54	6.06		
9	2835			4.02	5.50		
10	3150			3.61	5.06		
11	3465			3.28	4.70		
12	3780			3.00	4.40		
2	1750					14.53	15.96
3	2625					8.43	9.63
4	3500					5.98	7.10
5	4375					4.65	5.71
6	5250					3.82	4.83
7	6125					3.24	4.22
8	7000					2.82	3.76
9	7875					2.49	3.42
10	8750					2.24	3.14
11	9625					2.03	2.92
12	10500					1.86	2.73



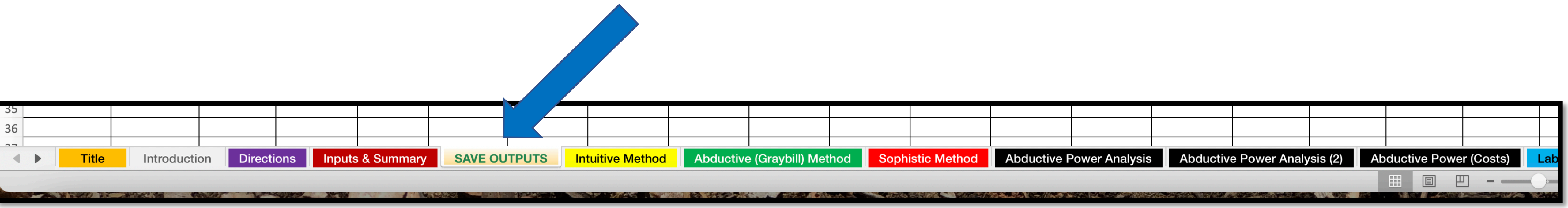
The number of replicates to graph is arbitrarily chosen. Here 3, 10 & 30 reps were chosen.



A 5% SEM:
Could not be found with 3 reps per pen.
Would cost $3200 with 10 reps per pen.
Would cost $5000 with 30 reps per pen.



A 3% SEM:
Could not be found with 3 reps per pen.
Might be found with 10 reps per pen.
Would cost $8,600 with 30 reps per pen.



Anyone can modify & improve the Excel workbook. Here a spreadsheet called "SAVE OUTPUTS" was created.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	**x Variable Name**	**y Variable Name**	**Intuitive Method**		**Abductive Method**		**Linear**	**Log10(x)**	**LN(x)**	**Sq Rt(x)**	**1 / x**	**x^2**	**$1/x^2$**	**Sq Rt(x+.5)**	**Ln(x+1)**	**Cube Rt (x)**	**ln[x/(1-x)]**	**0.5ln [(1+x)/(1-x)]**	**$x+x^2$**
2			**Mean**	**SEM**	**Mean**	**SEM**	**Coefficients of Determination = R^2**												
3	**Intake**	**Response**	**71.645641**	**10.4983036**	**71.64564**	**4.0719174**	0.5152	0.6124	0.6124	0.5647	0.6861	0.4254	0.7213	0.5641	0.6100	0.5810	0.6085	0.5148	0.4263
4																			
5																			
6	Diet Zn	Tibia Zn	71.645641	10.4983036	71.64564	4.0719174	0.515	0.612	0.612	0.565	0.686	0.425	0.721	0.564	0.610	0.581	0.609	0.515	0.426
7																			
8																			

Values from the "Inputs & Outputs" and "Transformations" Spreadsheets are written to the new spreadsheet. The results from Row 3 are from formulas. They can be copied and pasted special, values, to have a permanent record

Conclusions About Calibration Curves

- Practically everyone accepts the inverse prediction model because it is very intuitive
- Practically everyone misses the important point that y=*f*(x) does not necessarily give the same line as x= *f*(y).
  - Predicting x from the y=f(x) line gives an incorrect answer.
- When $r^2 > 0.95$ it doesn't matter much.
- When $r^2 < 0.95$ there may be distinct advantages to using an abductive method.
  - The ci of test samples is better approximated.
  - Ci's of samples near the center of the standard curve won't be overestimated.
  - Ci's of samples near the extremes of the standard curve won't be underestimated.

General Guidelines for Calibration Curves

1. Use at least 6 levels of x for your calibration curve
2. Center the calibration curve on values you expect from your test samples
3. For best results, try and use only a linear range of the response
4. Use the Abductive Method Equation to determine the amount of resources you can use for your:
    1. Calibration Curve
    2. Number of replicates for the unknown
    3. Economical improvement in CI
5. Use the Abductive Method Equation to determine the CI of the estimated content of the samples
6. Determine if a transformation will be helpful