This would be your first step, for example, when comparing data from sample measurements versus controls. One wants to know if there is any difference in the means.

Comparison of Standard Deviations

Table 4-2 Measurement of HCO_3^- in horse blood[a]

	Original instrument	Substitute instrument
Mean ($\bar{x}$, mM)	36.14	36.20
Standard deviation (s, mM)	0.28	0.47
Number of measurements (n)	10	4

a. Data from M. Jarrett, D. B. Hibbert, R. Osborne, and E. B. Young, *Anal. Bioanal. Chem.* **2010**, *397*, 717.

Is *s* from the substitute instrument "significantly" greater than *s* from the original instrument?

F test (Variance test)

$$F = \frac{s_1^2}{s_2^2}$$

If $F_{calculated} > F_{table}$, then the difference is significant.

Make $s_1 > s_2$ so that $F_{calculated} > 1$

Table 4-3 Critical values of $F = s_1^2/s_2^2$ at 95% confidence level

Degrees of freedom for s_2	Degrees of freedom for s_1: 2	3	4	5	6	7	8	9	10	12	15	20	30	∞
2	19.0	19.2	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.5
3	9.55	9.28	9.12	9.01	8.94	8.89	8.84	8.81	8.79	8.74	8.70	8.66	8.62	8.53
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.75	5.63
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.50	4.36
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.81	3.67
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.58	3.51	3.44	3.38	3.23
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.08	2.93
9	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.86	2.71
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.84	2.77	2.70	2.54
11	3.98	3.59	3.36	3.20	3.10	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.57	2.40
12	3.88	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.47	2.30
15	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.25	2.07
20	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.04	1.84
30	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.84	1.62
∞	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.46	1.00

For n observations, degrees of freedom = $n - 1$. There is a 5% probability of observing F above the tabulated value.

You can compute F for a chosen level of confidence with the Excel function FINV (Probability,Deg_freedom1,Deg_freedom2). The statement "=FINV(0.05,7,6)" reproduces the value F = 4.21 in this table.

$F_{calculated} = (0.47)^2/(0.28)^2 = 2.8_2$ $F_{calculated}\ (2.8_2) < F_{table}\ (3.63)$

Therefore, we reject the hypothesis that s_1 is signficantly larger than s_2. In other words, at the 95% confidence level, there is no difference between the two standard deviations.

Hypothesis Testing

Desire to be as accurate and precise as possible. Systematic errors reduce accuracy of a measurement. Random error reduces precision.

The practice of science involves formulating and testing hypotheses, statements that are capable of being proven false using a test of observed data. The **null hypothesis** typically corresponds to a general or default position. For example, the null hypothesis might be that there is no relationship between two measured phenomena or that a potential treatment has no effect.

In statistical inference of observed data of a scientific experiment, the **null hypothesis** refers to a general or default position: that there is no relationship (no difference) between two measured phenomena, or that a potential medical treatment has no effect. Rejecting or disproving the null hypothesis – and thus concluding that there are grounds for believing that there is a relationship between two phenomena (there is a difference in values) or that a potential treatment has a measurable effect – is a central task in the modern practice of science, and gives a precise sense in which a claim is capable of being proven false.

This would be the second step in the comparison of values after a decision is made regarding the F –test.

Comparison of Means

t test for comparison of means:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

This *t* test is used when standard deviations are not significantly different.!!!

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}}$$

s_{pooled} is a “pooled” standard deviation making use of both sets of data.

If $t_{calculated} > t_{table}$ (95%), the difference between the two means is statistically significant!

Comparison of Means

This *t* test is used when standard deviations are significantly different!!!

$$t_{calculated} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{(s_1^2/n_1) + (s_2^2/n_2)}}$$

$$\text{degrees of freedom} = \frac{\left[(s_1^2/n_1) + (s_2^2/n_2)\right]^2}{\frac{(s_1^2/n_1)^2}{n_1 - 1} + \frac{(s_2^2/n_2)^2}{n_2 - 1}}$$

Round the degrees of freedom from Equation 4-8 to the nearest integer.

If $t_{calculated} > t_{table}$ (95%), the difference between the two means is statistically significant!

Grubbs Test for Outlier (Data Point)

Mass loss (%): 10.2, 10.8, 11.6 (Sidney) 9.9, 9.4, 7.8 (Cheryl) 10.0, 9.2, 11.3 (Tien) 9.5, 10.6, 11.6 (Dick)

Cheryl's value 7.8 looks out of line from the other data. A datum that is far from the other points is called an *outlier*. Should the group reject 7.8 before averaging the rest of the data or should 7.8 be retained?

We answer this question with the **Grubbs test**. First compute the average ($\bar{x}$) and the standard deviation (s) of the complete data set (all 12 points in this example):

$$\bar{x} = 10.16 \qquad s = 1.11$$

Then compute the Grubbs statistic G, defined as

Grubbs test:

$$G = \frac{|\text{questionable value} - \bar{x}|}{s} \tag{4-9}$$

Table 4-6 Critical values of G for rejection of outlier[a, b]

Number of observations	G (95% confidence)
4	1.463
5	1.672
6	1.822
7	1.938
8	2.032
9	2.110
10	2.176
11	2.234
12	2.285
15	2.409
20	2.557

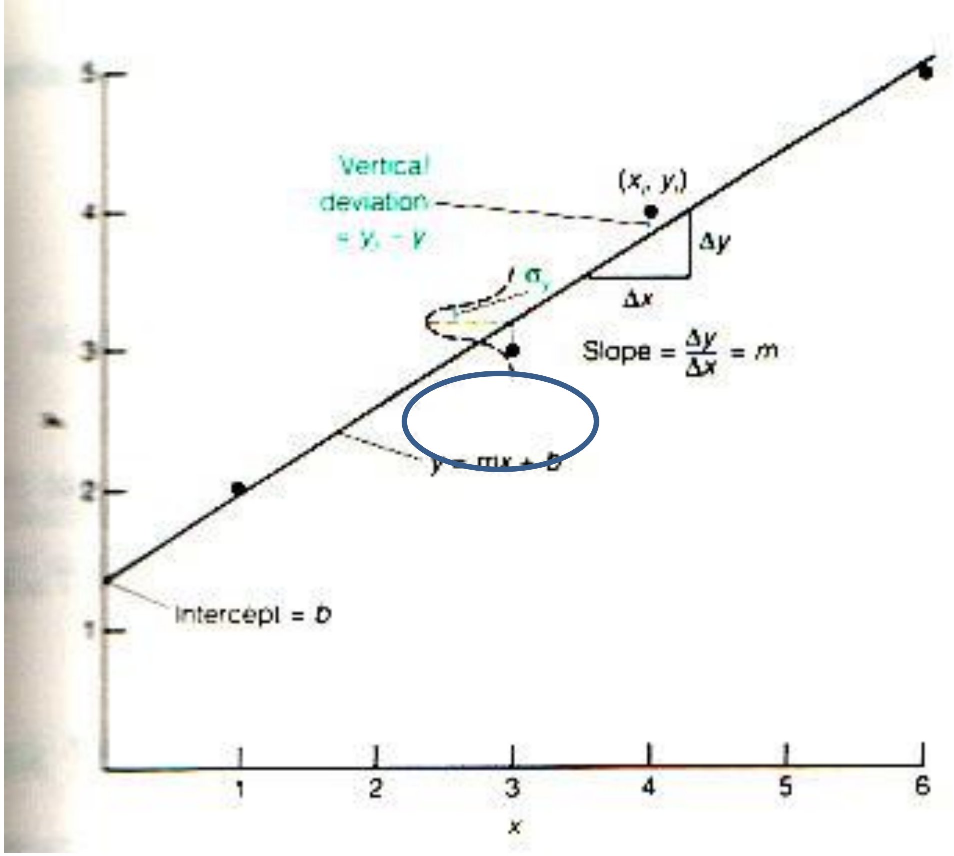
If $G_{calculated} > G_{table}$, then the questionable value should be discarded!

$G_{calculated} = 2.13$ G_{table} (12 observations) = 2.285

Value of 7.8 should be retained in the data set.

Linear Regression Analysis

The method *of least squares* finds the "best" straight line through experimental data.



Linear Regression Analysis

Table 4-7 Calculations for least-squares analysis

x_i	y_i	x_iy_i	x_i^2	$d_i(= y_i - mx_i - b)$	d_i^2
1	2	2	1	0.038 462	0.001 479
3	3	9	9	−0.192 308	0.036 982
4	4	16	16	0.192 308	0.036 982
6	5	30	36	−0.038 462	0.001 479
$\Sigma x_i = 14$	$\Sigma y_i = 14$	$\Sigma(x_iy_i) = 57$	$\Sigma(x_i^2) = 62$		$\Sigma(d_i^2) = 0.076\,923$

Quantities required for propagation of uncertainty with Equation 4-19:

$\bar{x} = (\Sigma x_i)/n = (1 + 3 + 4 + 6)/4 = 3.50 \quad \bar{y} = (\Sigma y_i)/n = (2 + 3 + 4 + 5)/4 = 3.50$

$\Sigma(x_i - \bar{x})^2 = (1 - 3.5)^2 + (3 - 3.5)^2 + (4 - 3.5)^2 + (6 - 3.5)^2 = 13$

Least-squares slope:

$$m = \frac{n\Sigma(x_iy_i) - \Sigma x_i \Sigma y_i}{D}$$

Least-squares intercept:

$$b = \frac{\Sigma(x_i^2)\Sigma y_i - \Sigma(x_iy_i)\Sigma x_i}{D}$$

where the denominator, D, is given by

$$D = n\Sigma(x_i^2) - (\Sigma x_i)^2$$

Variability in *m* and *b* can be calculated. The first decimal place of the standard deviation in the value is the last significant digit of the slope or intercept.

Use Regression Equation to Calculate Unknown Concentration

y (background corrected signal) = m x (concentration) + b

x = (y - b)/m

$$\text{uncertainty in } x\ (= s_x) = \frac{s_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \sum (x_i - \bar{x})^2}}$$

Report x ± uncertainty in x

s_y is the standard deviation of *y*.
k is the number of replicate measurements of the unknown.
n is the number of data points in the calibration line.
y (bar) is the mean value of *y* for the points on the calibration line.
x_i are the individual values of *x* for the points on the calibration line.
x (bar) is the mean value of *x* for the points on the calibration line.