This document has been prepared where possible using guidelines provided by the British Dyslexia Association.
The purpose of this presentation is to show a case study of a simple experimental design and statistical analysis based on the measurement of porcine blood plasma glucose.

The study is based on a Part 1 module from the BSc Animal Science degree course at the University of Reading.
Teaching Aims
Teaching Aims

• To teach skills relating to the planning of a basic laboratory assay.

• To demonstrate the need for a risk assessment when undertaking any form of experimental work where hazardous chemicals or other risks are present.

• To demonstrate the construction of a standard curve from which to calculate values from observed measurements.
Teaching Aims

• Data analysis to look at the Standard Error of the Mean (SEM) and Analysis of Variance (ANOVA).
Key Points

- Experimental Design
- Risk Assessment
- Standard Curve
- Standard Error of the Mean
- Analysis of Variance (ANOVA)
Other Study Examples

- While this case study focuses on glucose level in animal plasma, the same principles could be used for a number of different studies requiring the formation of a standard curve, for example:

- Suitability of soil for growing different crops by measuring the pH.

- Maturity of grain through the measurement of starch levels.
Background
In animal nutrition trials, it is often the case that the effect of different feedstuffs is monitored in a number of ways, for example:

- Weight
- Percentage body fat
- Blood composition

The purpose of this study is to calculate the concentration of glucose in animal blood plasma as a result of different feeding regimes.
Experimental Design
Feeding Regimes

• In this study, a number of piglets were split into four groups and fed with the following dietary supplements:
  – Group 1: Control diet (normal feed)
  – Group 2: Control diet with Ca salts of palm oil.
  – Group 3: Control diet with free C16:0 fatty acid.
  – Group 4: Control with linseed oil.

• The animals were fed for one month before 14 randomly selected individuals from each group were identified for measurement.
Whole blood samples were taken (Note: procedures such as this need to be performed under Home Office License).

The blood was collected in a vacutainer containing EDTA to prevent clotting.

Sample was spun in a centrifuge and the plasma removed by pipette and stored at -20ºC.

Prior to use, the sample was thawed slowly.
Risk Assessment
Assessing Risk

• **Before** undertaking any potentially hazardous activity, a risk assessment should be made.

• Although this example is focusing on a laboratory procedure, risk assessments are important for any potentially hazardous activity, for example:
  - Handling machinery
  - Working at heights
  - Working with hazardous chemicals
  - Heavy lifting
  - Working in noisy or dusty environments
An example of a risk assessment form is shown on the next slide (You should check with your safety officer for the form specific to your site).

- The top of the form identifies who will be undertaking the activity and where.

- **Section 1** identifies ALL of the potential risks in the given area / activity.

- **Section 2** describes the risk in more detail, explains any control measures already in place to reduce risk and identifies any further action required to reduce risk.
## AREA HEALTH AND SAFETY RISK ASSESSMENT FORM

<table>
<thead>
<tr>
<th>Assessment Reference No.</th>
<th>Area or activity assessed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment date</td>
<td></td>
</tr>
<tr>
<td>Persons who may be affected by the activity (i.e. are at risk)</td>
<td></td>
</tr>
</tbody>
</table>

### SECTION 1: Identify Hazards

- Consider the activity or work area and identify if any of the hazards listed below are significant (tick the boxes that apply).

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fall of person (from work at height)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Fall of objects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Slips, Trips &amp; Housekeeping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Manual handling operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Display screen equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Lighting levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Heating &amp; ventilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Layout, storage, space, obstructions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Welfare facilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Electrical Equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Use of portable tools / equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Fixed machinery or lifting equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Pressure vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Noise or Vibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Fire hazards &amp; flammable material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Vehicles / driving at work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Outdoor work / extreme weather</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Fieldtrips / field work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>Radiation sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>Work with lasers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>Hazardous fumes, chemicals, dust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>Hazardous biological agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Confined space / asphyxiation risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Condition of Buildings &amp; glazing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>Food preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>Occupational stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>Violence to staff / verbal assault</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Work with animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Lone working / work out of hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>Other(s) - specify</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SECTION 2: Risk Controls

For each hazard identified in Section 1, complete Section 2.

<table>
<thead>
<tr>
<th>Hazard No.</th>
<th>Hazard Description</th>
<th>Existing controls to reduce risk</th>
<th>Risk Level (tick one)</th>
<th>Further action needed to reduce risks (provide timescales and initials of person responsible)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>Med</td>
</tr>
</tbody>
</table>

Name of Assessor(s): SIGNED

Review date: Number of continuation sheets used:
Examples of Risk

In the laboratory case study, some examples of risk could be:

- Exposure to chemicals – risk should be assessed using the material safety data sheets (MSDS) for each chemical. Personnel are provided with adequate personal protective equipment (e.g. lab coat, gloves, safety specs) as required.

- Emergency escape route: exit routes clearly signed and accessible (i.e. doors not blocked).

- Electrical equipment: has it been fully safety tested?

- Fall of objects, for example boxes stored on high shelves.
Laboratory Procedure
Background

- The following slides show the chemical reaction that is utilised in this assay.

- While it is not necessarily important to understand the chemical reaction, it does give a good background to the mechanism by which a colour is achieved and how measurement of this colour relates back to the glucose concentration in the original sample.
Biochemistry

The determination of glucose in porcine plasma samples uses an enzymatic assay reagent containing glucose oxidase peroxidase and o-Dianisidine:

1. Glucose is oxidised to gluconic acid and hydrogen peroxide

\[
\text{D-Glucose} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{D-Gluconic Acid} + \text{H}_2\text{O}_2
\]
2. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a coloured product.

\[
\text{H}_2\text{O}_2 + \text{reduced o-Dianisidine (colourless)} \xrightarrow{\text{Peroxidase}} \text{Oxidised o-Dianisidine (brown)}
\]

3. Oxidised o-Dianisidine reacts with sulphuric acid to give a more stable coloured product which can be measured and used to determine concentration.

\[
\text{Oxidised o-Dianisidine (brown)} \xrightarrow{\text{H}_2\text{SO}_4} \text{Oxidised o-Dianisidine (pink)}
\]
Colour Measurement

- In order to use the colour change of o-Dianisidine it is necessary to be able to accurately measure the colour in order to relate it back to glucose concentration.

- The device used to measure the colour is a spectrophotometer or colorimeter.

- Given the number of samples, it is more convenient to use a plate reader that can measure multiple samples on one plate.
Plate Reader
The equipment listed below is specifically for the glucose assay described in this case study. Other experiments may require different equipment.

- Reagents
- Pipettes + tips
- Sample tubes (one per test subject) + rack
- Water bath set at 37°C
- 96-well plate
- Spectrophotometer or colorimeter capable of measuring at 540nm with plate reader
- Personal protective equipment (lab coat, goggles and gloves)
Experimental Method

• Mark up your tubes with the number of the sample you are going to analyse.

• Pipette in 0.5ml of each sample into a fresh tube – remember to use a different tip for each fresh sample to ensure no cross-contamination occurs.

• Start the reaction by adding 1.0ml of assay reagent using a pipette.

• Place the tubes in the water bath at 37°C for 30 minutes.
Experimental Method

• Carefully remove the tube from the water bath and place in the rack.

• Stop the reaction after exactly 30 mins by adding 1.0ml 6M H$_2$SO$_4$ and mix thoroughly.

• Remove 100μl from each tube and carefully pipette into a well on the 96 well plate.

• Read the plate at 540nm and calculate the glucose concentration from the standard curve.
Experimental Method
Standard Curve

• In order to understand the results, before running the test samples, a standard curve needs to be established.

• A standard curve is a set of samples with a known concentration of the material under study (in this case glucose) which can be run under the same experimental conditions.

• The results of the known concentration samples are plotted as a graph showing concentration (in μl/ml) as a function of (in the case of this example) absorbance at 540nm.
**Standard Curve**

**Equation for best fit**

\[ y = 141.6x - 6.5707 \]

**Indication of how ideal line is**

**Known value** (this is the determined value for actual samples)

**Measured value**
Standard Curve

• The value of $R^2$ as calculated on a spreadsheet such as Microsoft® Excel™ gives an idea of how ideal the best fit line is. The closer $R^2$ is to 1.000, the better the fit of the line is.

• The equation of the line allows for experimental results to be converted into concentrations.

• In the experiment, values for absorbance are measured and converted into glucose concentrations ($y$) by using the equation generated for the best-fit line on the standard curve or can be estimated by simply reading off of the graph. The best-fit equation is the more accurate method.
Control Samples

• There will always be slight differences each time an experiment is run so it is important that on EVERY plate that is read, a standard curve is generated and control samples (used on every plate) are run.

• The use of control samples allows for any differences between experiments run at different times (e.g. with different batches of reagents with slightly different concentrations or efficacies) or where there are variations in environmental conditions to be understood.
Replication

• Ideally, multiple replicates of each sample are run to get an average. In this case study, each sample is measured twice.

• For practical reasons, duplicates or triplicates may be taken and the reliability of the results can be checked by looking at the standard deviation of the replicated results.

• Ideally, the standard deviation should be as close to zero as possible, indicating that the results are close together and therefore reliable. If the standard deviation is high, it may be necessary to measure further replicates to get better confidence in the accuracy of the result.
Calculation of Standard Deviation

‘Standard deviation (SD) is a measure of spread which may be regarded as an average of the deviations of the observations from the arithmetic mean. It is equal to the square root of the variance.’

• A low value of standard deviation implies that the samples are all close to the mean whereas a high SD means that the data varies considerably around the mean.

• If the individual sample data points in a population follow a normal distribution around the mean, SD can be used to show the variability of dispersion of the sampled data.
Normal Distribution

• The following slide shows a normal distribution curve and the percentage of the data points that would appear within 1, 2 and 3 standard deviations around the mean (the central peak of the graph).

• The greater the standard deviation, the further away from the central peak the spread of sampled data is.
Normal Distribution

SD = Standard Deviation

3 x SD
2 x SD
1 x SD
1 x SD
2 x SD
3 x SD
Calculation of Standard Deviation

Using our data set from the calculation of means: 22, 25, 24, 28, 25 and 26, (assuming it is a sample from a larger normal population) first, we calculate the difference each point is from the mean and square the result:

\[(x_1 - \bar{x})^2\]

\[(22-25)^2 = (-3)^2 = 9\]
\[(25-25)^2 = (0)^2 = 0\]
\[(24-25)^2 = (-1)^2 = 1\]
\[(28-25)^2 = (3)^2 = 9\]
\[(25-25)^2 = (0)^2 = 0\]
\[(26-25)^2 = (1)^2 = 1\]
Calculation of Standard Deviation

Next, we divide the sum of these values by the number \( n \) of values minus 1 (or \( n \) if the sample is the entire population) and take the square root. This gives the standard deviation.

\[
\sqrt{\frac{9 + 0 + 1 + 9 + 0 + 1}{6 - 1}} = \sigma \text{ (or } s \text{)} = 2
\]

\[
s = \sqrt{\frac{1}{n-1} \sum (x_1 - \bar{x})^2}
\]
Calculation of Standard Deviation

• Once again, it is usually quicker to use the formula for standard deviation on a spreadsheet such as Microsoft® Excel™

• Calculation would use the STDEV or STDEVP functions (for either sample or entire population).
Standard Deviation

The table below shows the standard deviation for a few select measurements from the glucose assay.

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Absorbance at 540nm</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>8786</td>
<td>3.533</td>
<td>0.212839</td>
</tr>
<tr>
<td>8786</td>
<td>3.834</td>
<td></td>
</tr>
<tr>
<td>283</td>
<td>3.603</td>
<td>0.053033</td>
</tr>
<tr>
<td>283</td>
<td>3.528</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>3.617</td>
<td>0.033234</td>
</tr>
<tr>
<td>136</td>
<td>3.57</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>3.548</td>
<td>0</td>
</tr>
<tr>
<td>38</td>
<td>3.548</td>
<td></td>
</tr>
<tr>
<td>277</td>
<td>3.707</td>
<td>1.621396</td>
</tr>
<tr>
<td>277</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
Standard Deviation

• The results for animal number 38 show a standard deviation of zero indicating that the values are exactly the same.

• For animal numbers 283 and 136 the standard deviation is low indicating that the measured values are close together and probably reliable.

• For animal numbers 8786 and 277 the standard deviation is relatively high and these are examples where further replicates may be desirable.
Data Manipulation
Raw Data

- Raw data for the glucose assay study please open the following file:
  
  **Raw Data and Standard Curves.xls**
  *(Microsoft® Excel™ 97-2003 format)*

- Alternatively you can use data you have collected yourself.
Study Task #1

1. Using the data sets provided, generate the standard curve for each plate by plotting the known concentration versus the measured absorbance.

2. Use the standard curve to calculate the glucose concentration values from the absorbance values measured for the test samples.

Click here to go to the worked example.
Once the concentrations have been calculated, the next task is to calculate whether there are any statistical differences between the four treatments.

In order to do this, the first thing to look at are the means for the values calculated in the four groups and to look at the standard error of the mean.

Once this has been done, an analysis of variance can be performed on the data.
Standard Error of the Mean

‘The Standard Error of the Mean (SEM) is the standard deviation of the sampling distribution of the mean; it is a measure of the dispersion of the sample means and of the precision of the sample mean as an estimate of the population means.’


- It gives an idea of variance within the sample (i.e. difference between individual values and the overall sample mean).
Analysis of Variance

‘Analysis of Variance (ANOVA) is a powerful collection of parametric statistical procedures for the analysis of data, essentially comparing the means of various groups of data. It relies on separating the total variation of a variable into its component parts which are associated with defined sources of variation.’


• ANOVA assumes:
  – Data is normally distributed
  – Samples are independent
  – Variance of the populations are equal
Calculating SEM and ANOVA

- SEM and ANOVA can be generated on a spreadsheet such as Microsoft® Excel™.

- From the ‘Data’ menu, select ‘Data Analysis’ which gives options for both ‘Descriptive Statistics’ (for Mean and Standard Error) and ‘ANOVA: Single Factor’.

- When prompted, select the data (which should be in columns, one for each treatment).
Study Task # 2

1. Calculate the mean and standard error for the concentrations worked out in each treatment.

2. Perform an ANOVA test on the data to see if there are any statistical differences between the four treatments. Click [here](#) to go to the worked example.
Worked Examples
Example # 1

The data for the worked example can be found in the following file:

Raw Data with Concentrations and Standard Curves.xls (Microsoft® Excel™ 97-2003 format)
Example # 1

• To generate the standard curve, the first thing that is required is to plot the measured absorbance values against the known concentrations.

• In Microsoft® Excel™ the two columns of data should be selected and then plotted as an x-y scatter graph (from the insert menu) without a line.
Best-fit Line

• To get the equation or line required to calculate the concentration values for the test data, a best-fit line needs to be plotted through the standard curve results.

• In Microsoft® Excel™ this can be achieved by clicking on a datum point on the x-y scatter graph, right clicking the mouse and selecting ‘Add Trendline’.
Best-fit Line

• For this data, the relationship should be linear so this should be selected on the ‘Trend/Regression Type’ box.

• To get the equation of the line shown on the graph and $R^2$ (the measure of how accurate the line is), check the boxes for ‘Display Equation on chart’ and ‘Display R-squared value on chart’ before clicking ‘Close’.
The following two slides show the standard curves as calculated for the two plates used in this experiment.

As can be seen by the values of $R^2$ (which are very close to 1 in both cases) the line is a very good fit. The line for Plate 1 with an $R^2$ value of 0.9968 is better than that for Plate 2 (0.9858).
Standard Curve – Plate #1

Plate 1 Standard Curve

\[ y = 141.6x - 6.5707 \]
\[ R^2 = 0.9968 \]
Standard Curve – Plate #2

Plate 2 Standard Curve

$y = 161.68x - 9.2634$

$R^2 = 0.9858$
Calculating Concentration

- The first method of calculating the value for concentration is to estimate it from the graph.
- Using the measured value of absorbance, draw a vertical line from the x-axis of the standard curve to the best fit line and then read horizontally to the y-axis to get the approximate value of concentration.
Estimating Concentration

Measured absorbance = 0.4

Estimated concentration ≈ 50 μl/ml

y = 141.6x - 6.5707

$R^2 = 0.9968$

Estimated concentration ≈ 50 μl/ml

Measured absorbance = 0.4
Calculating Concentration

• Alternatively, concentration can be more accurately calculated from the equation of the best-fit line.

• In the case of Plate #1 the equation is:

\[ y = 141.6x - 6.5707 \]

• Substituting the measured value for absorbance for \( x \) gives the concentration as 50.0693 \( \mu l/ml \)
Estimating Concentration

\[ y = 141.6x - 6.5707 \]
\[ R^2 = 0.9968 \]

Measured concentration =
\[ (141.6 \times 0.4) - 6.5707 = 50.0693 \, \mu l/ml \]

Measured absorbance = 0.4
Concentrations

- The calculated concentrations for the glucose assay study can be found in the following file: Raw Data with Concentrations and Standard Curves.xls (Microsoft® Excel™ 97-2003 format)

Alternatively they are shown on the next slide.

- If you click on a cell in which the concentration has been calculated, you will see the formula that has been used to make the calculation in the fx bar above the spreadsheet.
## Concentrations

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.05536</td>
<td>5.06952</td>
<td>5.23944</td>
<td>5.42352</td>
</tr>
<tr>
<td>5.16864</td>
<td>4.8996</td>
<td>5.05536</td>
<td>5.33856</td>
</tr>
<tr>
<td>5.26776</td>
<td>5.86248</td>
<td>4.72968</td>
<td>5.16864</td>
</tr>
<tr>
<td>5.06952</td>
<td>5.91912</td>
<td>4.94208</td>
<td>4.99872</td>
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<tr>
<td>5.44948</td>
<td>5.142288</td>
<td>5.2878</td>
<td>4.948272</td>
</tr>
<tr>
<td>5.271632</td>
<td>5.077616</td>
<td>5.352472</td>
<td>5.174624</td>
</tr>
<tr>
<td>5.271632</td>
<td>5.061448</td>
<td>5.2878</td>
<td>5.239296</td>
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<tr>
<td>5.352472</td>
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<tr>
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<td>5.02704</td>
<td>4.758</td>
<td>5.26776</td>
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<td>5.1828</td>
<td>4.85712</td>
<td>4.60224</td>
<td>5.38104</td>
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<tr>
<td>4.689584</td>
<td>5.62176</td>
<td>4.60224</td>
<td>5.33856</td>
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<td>4.624912</td>
<td>5.70672</td>
<td>4.72968</td>
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<td>5.239296</td>
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<td>5.174624</td>
<td>5.093784</td>
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<td>4.78632</td>
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<tr>
<td>4.29072</td>
<td>5.05536</td>
<td>5.45184</td>
<td>5.48016</td>
</tr>
</tbody>
</table>
Click **here** to return to the presentation
Example # 2

The data for the worked example can be found within the following file:

Raw Data with Concentrations and Standard Curves by Treatment.xls (Microsoft® Excel™ 97-2003 format)
Mean and SEM

- Using the ‘Descriptive Statistics’ tool in Microsoft® Excel™, the following values are calculated.

<table>
<thead>
<tr>
<th></th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.066728333</td>
<td>5.168639667</td>
<td>5.130501667</td>
<td>5.13932</td>
</tr>
<tr>
<td>SEM</td>
<td>0.066935412</td>
<td>0.071944203</td>
<td>0.059802311</td>
<td>0.048859931</td>
</tr>
<tr>
<td>Sample Count</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

- The data indicates that the values are similar across the treatments. These results would normally be presented on a graph as in the following slide.
Mean and SEM

Error bars = SEM
Columns = means
Using the ‘ANOVA: Single Factor’ tool in Microsoft® Excel™, the following values are calculated.

**Summary:**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>24</td>
<td>121.6015</td>
<td>5.066728</td>
<td>0.107528</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>24</td>
<td>124.0474</td>
<td>5.16864</td>
<td>0.124223</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>24</td>
<td>123.132</td>
<td>5.130502</td>
<td>0.085832</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>24</td>
<td>123.3437</td>
<td>5.13932</td>
<td>0.057295</td>
</tr>
</tbody>
</table>

**ANOVA:**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.132687</td>
<td>3</td>
<td>0.044229</td>
<td>0.471928</td>
<td>0.702586</td>
<td>2.703594</td>
</tr>
<tr>
<td>Within Groups</td>
<td>8.6222</td>
<td>92</td>
<td>0.09372</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8.754886</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The p-Value

- In the table above, the p-value has been highlighted. This value gives an indication of the significance of the result.

- Whether the result is significant or not will depend on the size of p. The larger the value of p, the generally less significant the result. For example, to be significant at the 1% level, p would need to be less than 0.01.

- The value of p in this experiment is very high and therefore will not be deemed as significant.
Conclusions

• The results indicate that there is no significant difference between the four treatments i.e. they have no significant effect on blood plasma glucose levels.
Conclusion

• In this case study, you will have experienced a simple laboratory assay design and considered factors such as risk assessment and the generation of a standard curve.

• With the data analysis you will have seen the basic principles of mean, standard error of the mean, standard deviation and the analysis of variance, and the sort of information they can provide about a sample.