Vocational Aqualabs - Vocational Generic Skills for Researchers

Experimental Design

Unit 3 - Designing Experiments

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Education and Culture DG
Lifelong Learning Programme

Designing experiments

Experimental variables

- **Independent variables** are manipulated or chosen by the experimenter (e.g. water flow rate, treatment concentrations, times intervals for growth measurement etc).
- **Dependent variables** are those responding to the independent variables i.e. those measured by the experimenter (e.g. growth rate of fish over time, treatment response etc).
- **Extraneous variables** are those unselected variables affecting the response (e.g. amount of sunlight each day). Extraneous variables that are systematically related to the independent variable (e.g. nitrate concentration of the water flowing into the pond / tanks etc) are termed "**confounding**" variables

Designing experiments

A good experiment requires **Randomisation**, **Replication** and **Controls** – because:

- Enormous variability of biological material and systems and from the conscious or unconscious **bias** of the experimenter.
- Any given **treatment** must be applied to several (**replicate**) experimental units and must be **randomly** allocated to the experimental units.
- The probability of any given unit receiving any given treatment must be equal.
- In order to detect changes attributable to treatments, suitable **controls** must be employed.

Randomisation

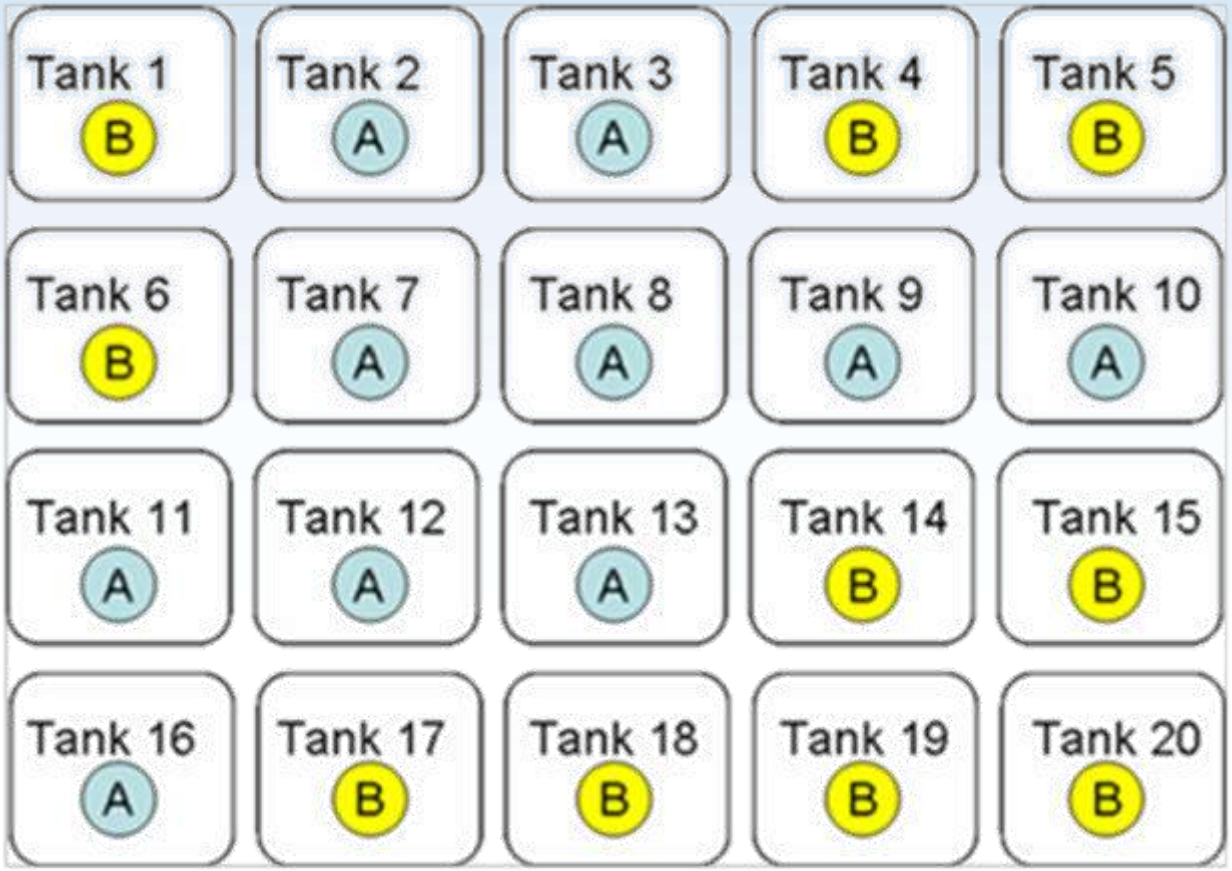
- Randomisation is an important factor in experimental design as it minimises the chance of a biased result through uncontrollable variability in the experimental equipment or materials.
- However, randomisation of an experiment is not always as straight forward as it may appear.
- Suppose we have two treatments, “Treatment A” and “Treatment B” and we have 20 tanks of fish divided into two groups of 10. The following gives a method for randomising these for use in the two experimental treatments:

Randomisation

- Randomly allocate the two treatments to the twenty tanks using a random number generator on a calculator.
- The calculator produced the following numbers

10	Assign to Tank 10
27	Too large, so 27 – 20 = 7 (Tank 7)
53	Too large, so 53 – 20 – 20 = 13 (Tank 13)
96	Too large, so 96 – 20 – 20 – 20 – 20 = 16 (Tank 16)
23	Too large, so 23 – 20 = 3 (Tank 3)
71	Too large, so 71 – 20 – 20 – 20 = 11 (Tank 11)
50	Too large, so 50 – 20 – 20 = 10 (Tank 10 already used)
54	Too large, so 54 – 20 – 20 = 14 (Tank 14)
36	Too large, so 36 – 20 = 16 (Tank 16 already used)
23	Number already appeared in the number series
54	Number already appeared in the number series
51	Too large, 51 – 20 – 20 = 11 (Tank 11 already used)
50	Number already used in the number series
14	Assign to Tank 14 but already used
28	Too large, so 28 – 20 = 8 (Tank 8)
02	Assign to Tank 2
12	Assign to Tank 12
29	Too large, so 29 – 20 = 9 (Tank 9)

Randomisation



Replicates

- Replicates are essential to good experimental design due to:
  - variability of biological material
  - unexpected and inexplicable changes in any experiment conducted in the real world-world.
- Replicates are critical for field experiments since fewer variables may be controlled

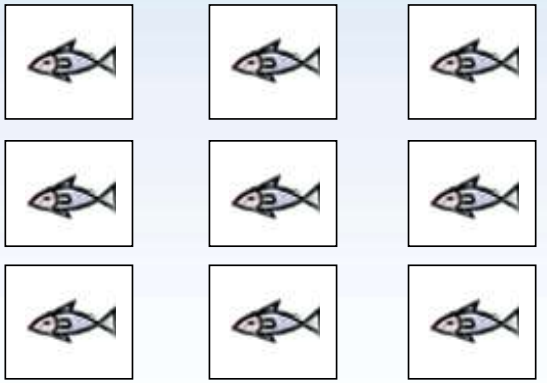
Replicates

- Ideally, as many replicates as possible
- Limited by feasibility and budgetary constraints?
- Rule of thumb –
  - At least 5 replicate groups
  - Bare min of 3

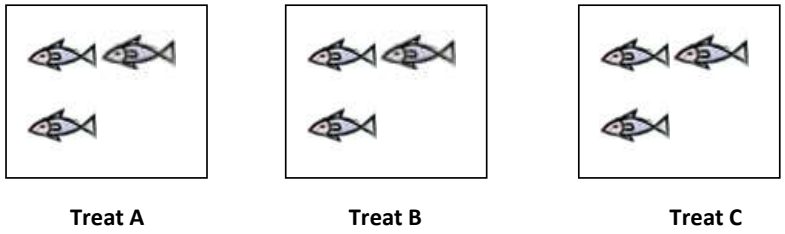
Pseudo-replication

- This is “the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not [truly] replicated (though samples may be) or replicates are not statistically independent” (Hurlbert, 1984)
- Which experiment is pseudo-replicated?

Experiment 1



Experiment 2



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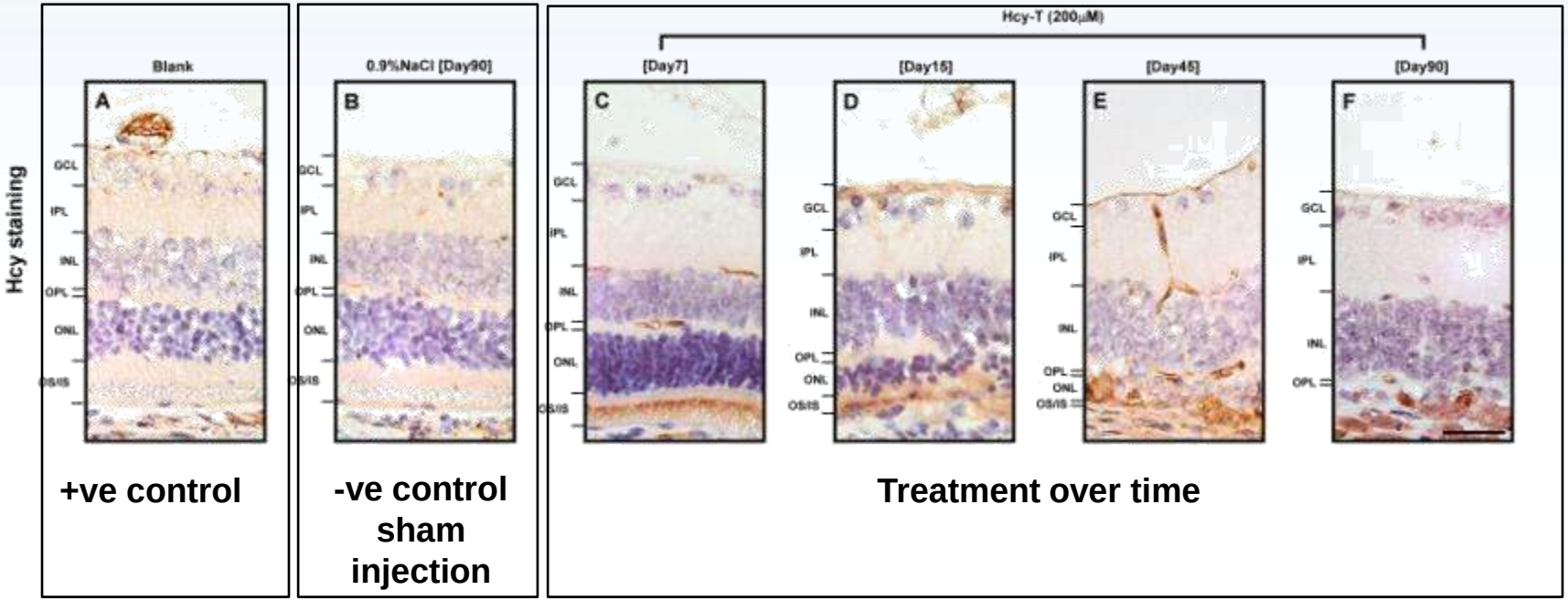
Controls

- Controls provide a baseline which allows assessment of changes due to experimental treatments.
- Controls MUST be treated identically in every respect to your test population, apart for the lack of the test condition itself, e.g.



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Sample size

The sample size determines:

- The ability of your experiment to resolve differences between population samples
- The likelihood that a sub-sample will accurately reflect the population it was taken from
- The statistical tests which may be employed to analyse your results, and the statistical power of the methods used.

Sample size

Sample sizes may be chosen in several different ways:

- **Expediency** – e.g. include those items readily available or convenient to collect. A choice of small sample sizes, though sometimes necessary, can result in wide confidence intervals or risks of errors in statistical hypothesis testing.
- Using **a target variance** for an estimate to be derived from the sample eventually obtained
- Using **a target for the power** of a statistical test to be applied once the sample is collected.

Sample size

- Larger sample sizes generally lead to:
  - Increased statistical precision (Law of large numbers)
  - Increased probability of normally distributed data (Central limit theorem)

Checklist – good design

The checklist should include:

- Select the sample group carefully to avoid confounding variables
- Randomly assign a treatment
- Try and make studies single or double blind, where:
  - First – Experimenter does not know from which individuals get which treatment
  - Second – Analyst does not know from which group a sample derives
- Random or Crossover design – for two treatments A and B some subjects are randomly assigned to treatment A initially and then move to treatment B and some *vice versa*.

Alternative Designs and Considerations

Power and pre-sampling

- The power of an experimental design is the probability of allowing one to correctly reject a false null hypothesis or accept a valid one.
- Depends on:
  - sample size employed,
  - the actual difference between the populations being studied,
  - the variability of the populations and the level of statistical significance that has been chosen.

Power and pre-sampling

- The power of an experimental design is the probability of allowing one to correctly reject a false null hypothesis or accept a valid one.
- Depends on:
  - **sample size employed**,
  - the actual differ[illegible]between studied,
  - the variability of the populatio[illegible] statistical significance that has

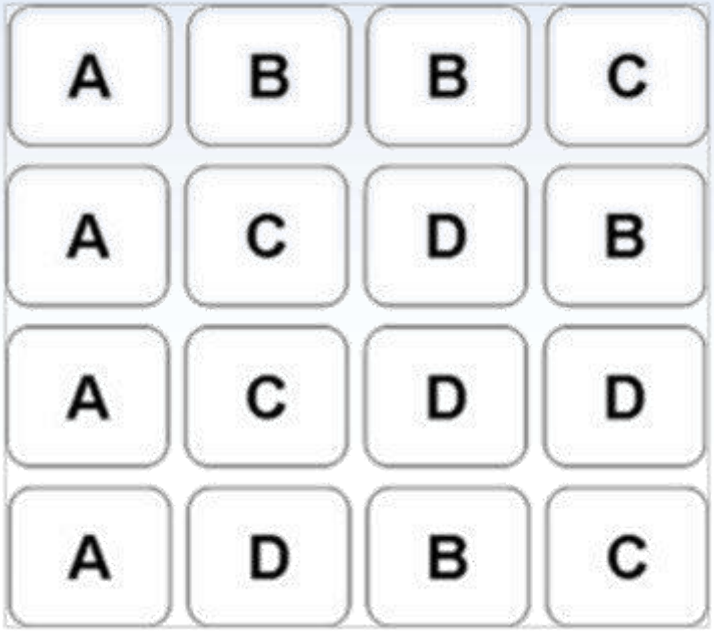
In practice, the easiest way to increase the power of an experimental design is to increase the sample size!

Sample size - again

- It is possible to calculate the sample size required to detect a given difference between populations.
- The difference may be estimated by pre-sampling or by using past studies as a guide.

Randomised Block Design

- Consider a standard randomised design?
- By random chance all treatment A's are within the first column
- What if there is a gradient of light coming from the left of the lab?



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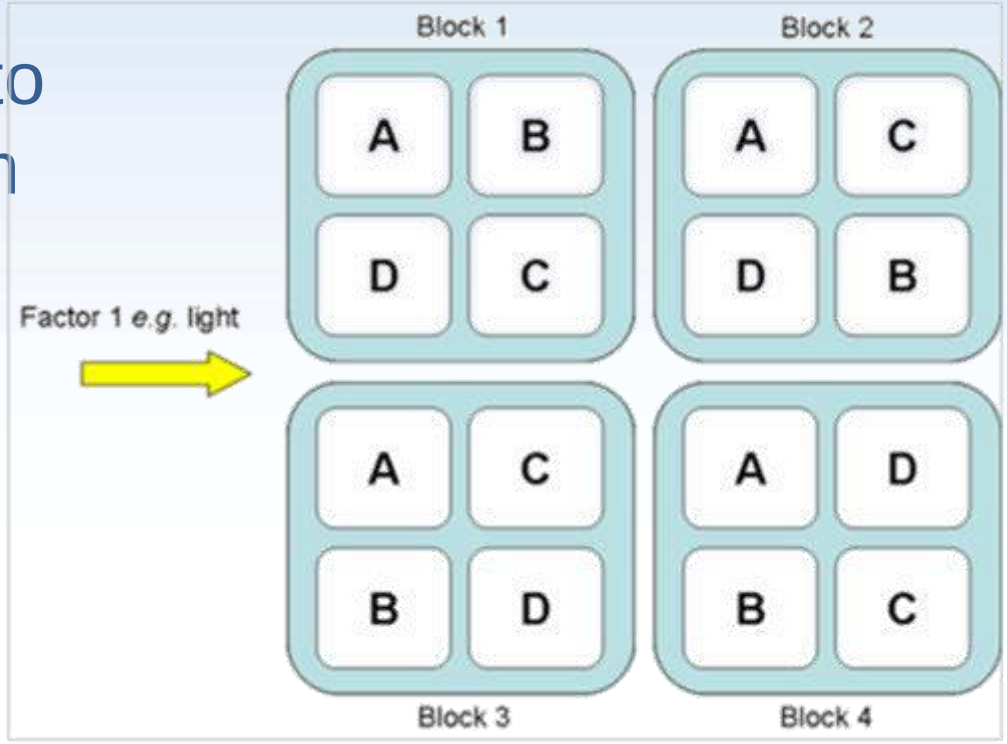
Randomised Block Design

- A randomised block design can be used to solve such a problem
- Each block receives one treatment randomly allocated
- Improved more...?



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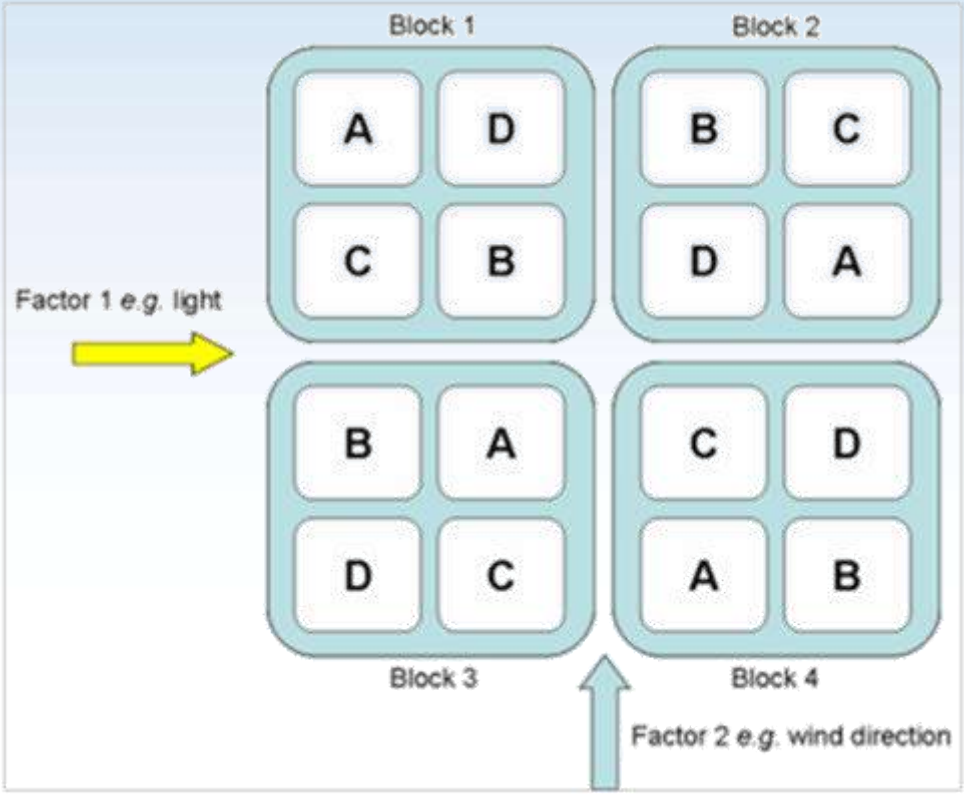
Latin Square Design

- Each block has one treatment

AND

- Each treatment also randomly allocated to each row and column
- Will allow for multiple confounding factors



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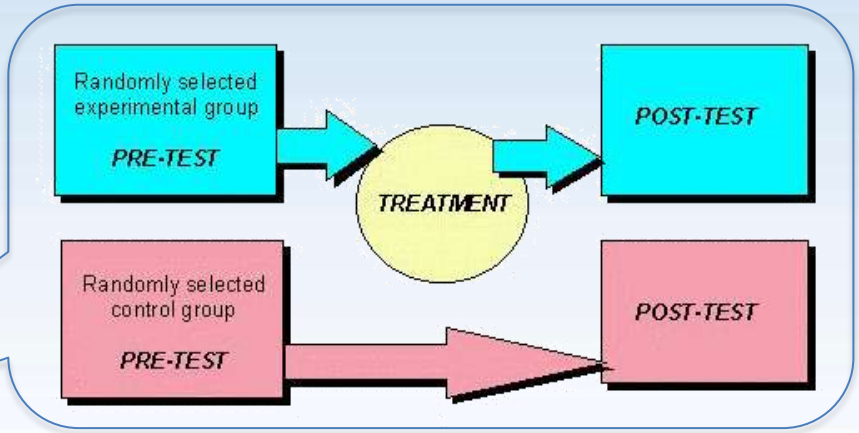
Factorial Design

- Used to examine the effects and interaction of two or more factors
- e.g. Different levels of lipid and protein in food; 3 x 4 factorial, 5 replicates
- Advantage – only need a single experiment as the hidden replication examines the interaction

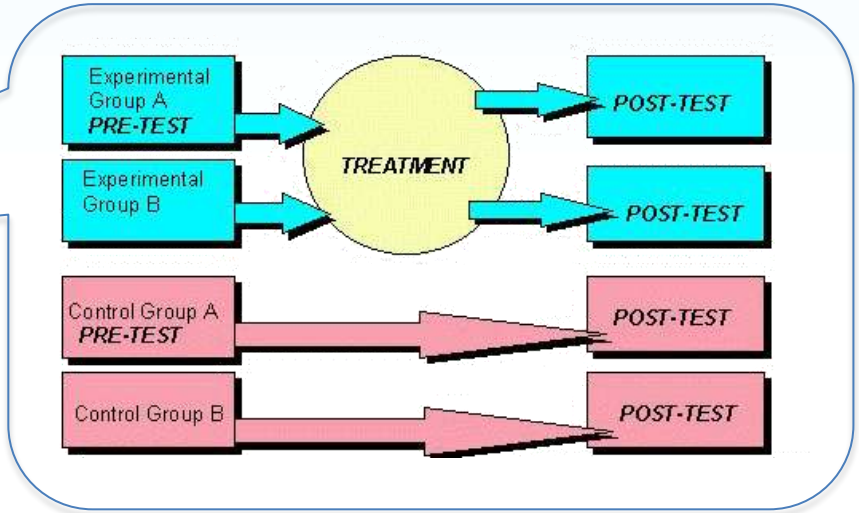
Protein	Lipid		
	Low	Medium	High
Low	5 reps	5 reps	5 reps
Medium	5 reps	5 reps	5 reps
High	5 reps	5 reps	5 reps
Very high	5 reps	5 reps	5 reps

Other Group designs

There are many other variations on such experimental designs – such as:



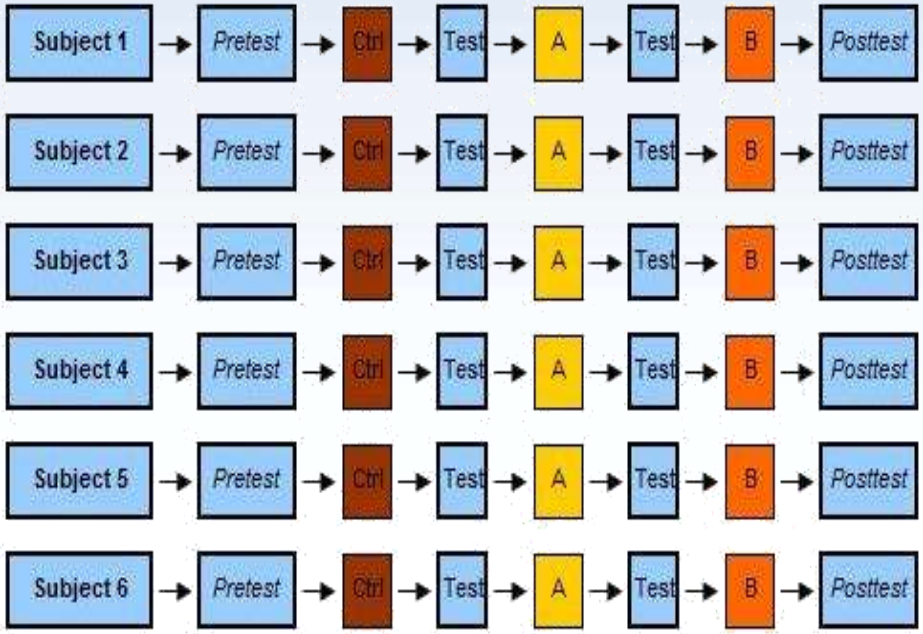
- Pre-Post Randomised Group design



- Solomon Four Group design

Repeated measures

- These designs are used if measurements or observations are made on the same sample/population a number of times over the study
- This must be used if measurements at each time point are being directly compared from time-point zero (temporal control)
- Use suitable statistical analysis i.e. Repeated measures ANOVA.



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Thank you

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