Enhancing Experimental Design: Minimizing Confounding Factors
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Abstract

Biological Question: The content of this module focuses upon refinement of the experimental design process and thus can be applied to a wide variety of biological courses. The activities developed for this module were developed so that it may be disseminated in a laboratory or classroom setting.

Statistical Content: This module was designed as a companion module to "Experimental Design" by Stuart and Rundell. In this module students will learn how to refine an experimental approach in order to remove the possibility of alternative solutions to a scientific question. This module is intended for students that have a basic understanding of experimental design concepts such as hypothesis generation, independent and dependent variables, controls, and use of statistics to evaluate data.

What students do: In this module students are introduced to experimental design methods to identify and control for confounding variables. Students will work in teams to conduct an experiment to determine which type of paper airplane is capable of the longest flight. At the conclusion of the experiment, the class will discuss variables present within the experimental process that confound the results of the experiment. After identifying confounding variables, students will work in teams to redesign the experiment so that conclusive statements can be made about the results.

Skills: Students will learn to identify confounding variables in an experimental design and employ experimental design methods to control for confounding variables.

Student-active approaches: Students will work in teams to conduct and evaluate an experiment and as an entire class to identify confounding factors.

Assessable outcomes: To assess student understanding, additional exercises are provided in the Faculty Notes section. These exercises can be assigned as additional in-class work, outside-of-class work, or as a quiz/exam question.

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Background

Goal of activity:
By the end of this activity students will be able to identify confounding variables and employ experimental design methods to control the impact of confounding variables in an experiment.

Basic Definitions and Principles:

Confounding variables: Comparative experimental design processes evaluate how an independent variable(s) impacts a dependent variable. Often when developing these types of experiments there may be confounding variables that are embedded in the experimental design process. The presence of confounding variables introduces the chance for alternative reasons (explanations) for a response (result) [2, 8]. In biological sciences the presence of large uncontrolled variations in experimental settings is common.

Perhaps one of the most famous examples of confounding is the Hawthorne effect. The Hawthorne effect was identified from a series of experiments conducted at the Hawthorne Works electric company in the Chicago area from 1924 to 1932. One of the studies conducted during this time was to examine the effects of lighting on worker productivity. Researchers found that regardless of the amount of lighting in the workplace there was an increase in worker productivity. After the study concluded, the company noticed that the level of productivity decreased. Results suggested that productivity was tied to the workers' impression of management's interest in their abilities, and as a result, no conclusions could be made on how the level of lighting impacted worker productivity [6].

One of the challenges for scientists when developing an experiment is identifying the possible confounding factors and determining their impact on the results. Some of the best examples of where scientists control for the presence of confounding variables are in clinical studies. For example, in drug studies, there are often subgroups (gender, age, race, etc.) within a study if the researchers feel that these factors influence the effect of the drug. Additionally, combinations of treatment options are typically used in clinical trials to identify the most effective treatment mode.

Experimental Design Methods to Control Confounding Variables: The two main options used to control confounding variables is to reduce the presence of a variable by "blocking" or to enhance the ability to evaluate the independent and dependent variable interactions by "factorial design" [7, 8].

There are two forms of blocking: Completely randomized block design (CRBD) and randomized block design (RBD). For CRBDs, there is a completely random assignment of test subjects to test groups. This process is typically only used on test objects in which there is minimal variability. For example in the module presentation, there is an example of pH test strips testing the pH of a solution. Since the test strips came from the same batch, it is assumed that the strips would be the same. Therefore, to run this test the test strips could be randomly assigned to different solutions.

RBD is more common in biological and clinical studies, in which there can be significant variability in living test subjects. A clinical trial RBD is presented in the module presentation in which a population of subjects is sub-grouped based upon specific characteristics [3, 7, 8]. In this process the researcher has created sub-groups that are similar in nature (gender, age, and amount of sleep). For RBDs to be effective the variability within each block, or sub-group, has to be less than the variability of the entire subject population.

Factorial experimental designs are used to explore the impact of multiple independent variables. Factorial designs evaluate "factors" which are independent variables at different "levels" or concentrations [8]. For example, in a drug metabolism study blood samples were collected at 1 minute, 10 minutes, and 30 minutes post injection. In this study, the factor is time which has three levels (1 minute, 10 minutes, and 30 minutes).

Setting up a factorial design will allow the researcher to know exactly how many experimental runs are needed to determine a result. Following the example provided in the module presentation on diet, exercise, and sleep the experimental design has 3 factors (diet, exercise, and sleep) in which each factor has 2 levels (diet= 1200 or 1500 calories/day, exercise = 30 minutes or 1 hour/day, and sleep = more than 8 hours or less than 8 hours/day). The factorial design is expressed as the number of levels for factor 1 X number of levels for factor 2 X ….. X number of levels for factor n. The factorial design for the previous example would be 2 X 2 X 2. Based on this information a total number of 8 experimental tests will need to be run to evaluate all possible combinations.

There are three main forms of factorial design: complete, fractional, and incomplete. A complete factorial design includes all possible experimental runs. This form is only run when there are not very many experimental runs required. The multiplicative nature of a complete factorial design may become both time and cost prohibitive. To address these challenges scientists use a fractional factorial design. The fractional factorial design follows the hierarchical ordering principle in which experimental runs that have three or more factors in play are not usually significant and thus can be ignored. Details on how a fractional factorial is developed can be found in Wu et al. [9]. More common in medicine is the use of the incomplete factorial design. This is a subset of a complete factorial design. What is unique with this form is that experimental runs that would provide information that scientists consider unhelpful or unethical would not be run. For example, if a new study was evaluating three drugs and it was shown that using all three drugs at one time was toxic to a patient, this experimental run would not be performed. Similarly, it would be unethical to have a placebo group in which cancer patients would receive no treatment [1, 7, 9].

Incomplete factorial designs are presented slightly different than fractional and complete factorial design. For example, researchers are conducting a study that is evaluating the effects of three new medications (A, B, and C) at 2 different concentrations to treat breast cancer. The researchers believe that taking medications A and C at the same time would be toxic to a patient. As a result this experimental run would be omitted from the experimental design. The proper notation for this incomplete factorial design would be $2^3 - y_{ac}$ [1].

<u>Module Activity Length</u>: 65 - 75 minutes; if running short on time, part 2 of the activity could be assigned as an outside of class assignment.

<u>Activity Part 1: (run time 40 minutes)</u>
Have students work in teams of four. Each team will need to be given two pieces of 8.5X11 inch paper and the instructions for the activity (Student Instructions Section). Instruct teams to determine which paper airplane model is capable of a longer flight. Using the provided directions the students must create the two described paper airplanes, run their flight tests, and then report on the board the length of flight for each model. After the testing have the students place planes in designated areas for model A and model B. *It is important during this session of the activity that you do not provide suggestions of a specific location to throw the planes (if possible) or how the planes are to be thrown.*

Next, have the class work together to determine the average flight length and standard deviation for each model. Then, have the class calculate the t-test result (set evaluation mark at $\alpha = 0.05$). After determining the statistical results have the teams evaluate the model A and B planes. Recommend to students that they should note differences and similarities among the planes representative of models A and B. If possible use a visual display system to project the planes on a screen so that the class can participate as a whole in the discussion. If that option is not available have the students view the planes and make observations in their groups.

After these observations, have the students break back into their teams to decide if they consider the student t-test results valid. After the discussion session, have each team share their opinion and reasons why they felt the results were valid or invalid. During the student discussions, write on the board the confounding variables that students note on the problems with the design.

If students do not acknowledge problems with making a sound evaluative judgment bring up concerns that you noticed. For example, re-examine the planes within a model type and identify differences in construction. Have students share how they launched the airplanes (location in the room, hand location on the airplane).

> *<u>A short list of confounding variables:</u>*
> *Airplanes within groups have variance in structure*
> *Consistency of launching airplanes within groups and among groups (hand placement, velocity of throw, angle of throw)*
> *Weight of paper (quality control) – weigh the airplanes*
> *Location of launch*
> *Airplane construction (tightness of the folds, accuracy of the folds)*

After identifying confounding variables have the students make recommendations on how some of the confounding variables could be minimized. List these recommendations on the board and then start the module presentation. Revisit these recommendations when discussing factorial design and blocking in the presentation to show how the student recommendations align with strategies to minimize effects of confounding variables.

<u>Presentation (run time 10 - 15 minutes)</u>
The presentation explores the use of factorial and block design to control for confounding variables in an experiment. This presentation addresses methods to employ when designing an experiment but does not delve into the theory on these methods. References used to develop the content of this module provide theoretical explanations of factorial and block design [1 – 5, 7-8]. References are listed at the end of this document. The PowerPoint slides of the presentation follow the reference section.

<u>Activity Part 2: (run time 10 - 20 minutes)</u>
After the presentation have the students work in teams to define which blocking and factorial factors they would consider to test the hypothesis that there is no difference in flight length between paper airplane model A and model B. If time permits have the teams share their experimental design with the class.

> *A completely randomized block design could be employed for the assignment of paper to be used to make paper airplane model A and B.*
>
> *There are a lot of options in the factorial design. In this instance, it would not be appropriate to run a complete factorial design due to the volume of confounding factors.*

Using the list from above some items can be somewhat controlled. The location of the launch can be decided upon and used as the sole place for testing.

Factor 1: Airplane model type (A or B) – this must be present in all experimental designs.
Factor 2: How the plane is launched. Determine a few methods to use as a launching method and then test. (Note: To truly standardize launch method a machine would probably be the best method to employ although not realistic for this module).
Factor 3: Tightness of the folds to the airplane.
Factor 4: Accuracy of the folds to the airplane

Possible Fractional Factorial Design:

Factor 1: Airplane model type
Factor 2: How plane is launched (at the nose, body, tail)

This would be a fractional 2 X 3 factorial design: (2 levels of factor 1) x (3 levels of factor 2).

Student Instructions

The Paper Airplane Experiment

Directions: Working in teams of 4, test the following:

Hypothesis: There is no difference in flight length between paper airplane model A and model B

Model A Paper Airplane [1]

1. Get an 8.5 X 11 inch piece of paper
2. Fold the paper in half along the length of the paper and then unfold
3. Fold the right corner of the paper towards the middle. The top right-hand corner of the paper should meet the middle crease.
4. Repeat step 3 for the left hand side of the paper
5. On the right side fold the top edge of the paper to the middle crease.
6. Repeat step 5 for the left side of the paper
7. Fold the paper in half along the middle crease created in step 2
8. Create wing on right side by folding the top of the paper down to meet the bottom of the body of the airplane
9. Report step 8 for the left side of the plane
10. Label plane with a “secret” symbol that will only be recognizable by your team
11. Throw airplane and record flight length on the board and associated “secret” symbol
12. Put airplane in area labeled Model A

Model B Paper Airplane [2]

1. Get an 8.5 X 11 inch piece of paper
2. Fold the paper in half along the width of the paper and then unfold
3. On each side of the paper fold each corner towards the center line
4. Turn the paper over and fold it in half along the centerline
5. On the right side of the plane fold the paper in half again
6. Repeat step 5 for the left side of the plane
7. Label plane with a “secret” symbol that will only be recognizable by your team (must be different from model A)
8. Throw airplane and record flight length on the board and associated “secret” symbol
9. Put airplane in area labeled Model B

References

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[2] “The Dart.” Internet: http://www.10paperairplanes.com/how-to-make-paper-airplanes/09-the-dart.html. Accessed 6/18/2012.

Faculty Notes

Objectives and audience: This module is intended for undergraduate students learning about experimental design. The aim of this module is for students to be able to develop experimental designs that reduce the impact of confounding variables in data results. The activity was designed for students to work in teams of 4 and as a whole class. Teams of 6 would be effective for the team work; however, it is not recommended to go larger than groups of 6.

Suggestions for using the exercise: For part 1 of the student activity, the instructor should provide very little guidance. Depending upon the location of the instruction it may be necessary to dictate where students may launch planes.

Assessment and evaluation: This module was designed for courses that have students practice experimental design in the course. In the additional activities section there are additional exercises that may be used to evaluate individual skill levels in this area. The exercises could be assigned as in-class work, out-of-class work, or used on a quiz or exam.

Presentation:

A few slides in the module presentation need further explanation than what is captured on the slide.

Details on those slides are provided below:

In slide 3 a short recap of the Hawthorne effect (presented in the background) could be presented to give students an example of how results can be confounded.

In slide 4, to describe confounding variables the following story could be used: I noticed when I walk my dog she always goes “crazy” when she walks past another dog. So, I decided to set up an experiment to determine if the presence of a dog while she is walking makes her bark. For the test, I ran 4 experimental runs in which I walked my dog past 4 different dogs. Then I walked her down the street 4 times in the absence of a dog. The results showed that my dog only barked when a dog was present. While this experiment may seem straightforward at first there are several confounding factors that may give rise to alternative explanations for dog barking. For example, I first ran 4 trials with the presence of a dog and then 4 trials in the absence of a dog. What if my dog was tired by the 6th experimental run? Also, I could be giving non-verbal feedback to the dog that is prompting the barking. Since I have decided she always barks when we pass another dog I may tighten my grip on the dog leash or shorten the leash. This nervous cue could be a trigger for the dog to bark and was not considered in the experiment design. Final take home note is that a good experimental design is crafted to minimize the opportunities for the presence of alternative explanations for a result.

Additional confounding factors to be explored in the example include: size of dog (big versus small), and the location of the experiment (are you at a location the dog has never been to versus one where it always walks?)

In slide 8: Have the students state what the factors and levels are for the experiment. This experimental design has 3 factors (diet, exercise, and sleep) in which each factor has 2 levels (diet= 1200 or 1500 calories/day, exercise = 30 minutes or 1 hour/day, and sleep = more than 8 hours or less than 8 hours/day).

Next, have the students describe the factorial design: The factorial design would be a 2 (diet option) X 2 (exercise option) X 2 (sleep option). A total of 8 experiments will need to be run to test all possible combinations.

Slide 9: In an experiment to determine the effect of antimicrobial solutions on shopping cart handles there are three different antimicrobial agents that will be tested plus a control. The effectiveness of each agent and control will be evaluated on the plastic and metal surfaces present on the handle of a shopping cart. Effectiveness of testing agents will be evaluated at 1, 10, 20, and 30 minutes post application. What is the factorial design?

The factorial design for this experiment would be: 4 (treatments) X 2 (test surfaces) X 4 (time interval). To run this experiment you would need to run 32 experimental conditions.

A special note for the challenge problem: This type of breakdown is defined by a classification of factors. Since the test surface is not a "treatment" that is changeable by the scientist but an intrinsic property of the shopping cart it would not be acknowledged as a "true" factor. To be more accurate in describing this factorial you would describe it as a 4 X 4 with a 2 X 1 classification of the units [2]. In which the units in this case would be the shopping cart surface (metal and plastic).

In slide 10, to explain incomplete factorial design to students describe how in clinical trials it may not be feasible or ethical to run a complete factorial design. For example, in a cancer treatment study, it would be unethical to have a group receive no treatment. An incomplete design may also be used in instances in which the researcher feels that gathering information on specific experimental runs would not advance the science [1].

Slide 12: Additional information to note when explaining the slide is that test strips removed from the box will be randomly assigned to a pH solution. This test is run under the assumption that pH test strips are standard within a box.

Slide 13: Use the following details to further describe the example of a randomized block design: For this experiment we want to determine if an energy drink helps to maintain high energy levels. To make sure that other variables such as gender and amount of sleep per night do not affect the results, we have created subgroups of the test population. When assigning people to placebo or energy drink group you would want an equal representation of each block within a test group.

<u>Additional Activities:</u> These exercises are useful to assess student ability to identify confounding variables and employ methods of experimental design remediation for confounding variables. These exercises could be used for in-class work, out-of-class work, or used on a quiz or exam. The exercises were designed to show a spectrum of the types of problems that can be constructed. These exercises can be readily adaptable to any course by telling a story about an event that requires experimental design. The text in gray italics provides some of the possible solutions to the posed problems.

Example Exercise 1:
Jacob was in charge of making 800 brownies for his family reunion. When he went to the grocery store he saw that in order to make that many brownies he would have to purchase two different brands: a

name brand and the store brand. He feels that the store brand will not taste as good as the name brand but does not have the time to go to another store to purchase more name brand brownie mix. Also during his shopping trip he purchased 10 cartons of eggs, 3 bottles of canola oil, and 20 aluminum foil baking pans, in which 10 pans were green in color and 10 pans were yellow in color. He was able to purchase the same brand for his eggs, oil, and pans. Having to make 800 brownies, Jacob starts baking as soon as he gets home. He decides that he will bake the name brand brownies in the green pans and the store brand brownies in the yellow pans. To expedite the baking process he bakes 4 pans of brownies at a time. In the first day, Jacob worked from 1 pm to 1 am to make 600 brownies. The next day, he baked from 10 am to 2 pm to make the remaining 200 brownies. At 3 pm Jacob left for his family reunion. At the reunion, he received mixed reviews on his brownies. Since he was with family they had no problem sharing their negative reviews on the brownies which included comments that the brownies tasted dry, were not very flavorful, and not completely done. He saw that most of the negative reviews came from people who had taken brownies from the yellow pans. To defend his brownie making prowess, he immediately blamed the store brand brownies for the negative reviews.

What would be the independent and dependent variables in an experiment to determine which brownie mix (brand name and store brand) results in better tasting brownies?

Independent variable: *brownie mix*
Dependent variable: *taste*

From the story above what are the confounding factors that would support alternative reasons for brownie taste?

There will be inherent variability in eggs, pans, and oil
What process was employed to make the brownies?
- *Were the eggs at the same temperature for each test?*
- *Did he make both mixes at the same time? Did he make one mix first and then moved to another?*
- *What order were the pans placed in the oven? Did he always place a certain brand in one location*

What was the temperature in the room? Was it consistent the entire baking process?
Were brownies allowed to cool the same amount of time for each batch?
Were the directions followed according to each brand?
Were the same utensils (bowl, mixing utensil) used to make both brownie brands?
Were the utensils cleaned and dried in-between each brownie mix?
When were the brownies made (Does the baker change behavior over time?)
Did the temperature in the over remain constant during the entire process?
Does the color of the baking dishes impact the brownie taste?

Which confounding factor(s) do you think need to be controlled to be able to make a judgment on which brownie mix is better?

Location in the oven for brownie mixture

Design a process that would allow for the determination of which brownie mix was better. The design should consider methods that minimize effects of confounding variables (blocking) and enhance detection of independent variable (brownie mix type) (Factorial).

Blocking:
Items that could be considered for blocking are the eggs, pans, and oil. While the same type of pans, oil, and eggs are used in the experiment there is the possibility of poor quality control. For example, one container of eggs may be slightly older/younger than another container. To control for this variability you could create sub groups based on egg carton. For each experimental run there would be representation of each group.

Factorial Design:
This design can be set up a variety of ways depending upon what the team identifies as the most impactful confounding variable. For this solution the baking location of the brownies in the oven is considered the most impactful confounding variable.

To address this issue we would run a 2 X 2 factorial design in which factor 1 would be pan location (top or bottom rack) and factor 2 would be brownie mix type (name brand or store brand) . This experiment could be expanded to a 2 X 2 X 2 factorial design if the side of the oven (right or left) was considered in the design.

Example Exercise 2

Dr. Brown created a new smoking cessation program that involves the use of pharmaceutical and group therapy. He is in the beginning stages and wants to make sure his regiment is the most appropriate method to help people stop smoking. He has already recruited 100 smokers to participate in the study. Describe the experimental design you would employ to optimize patient care for smoking cessation.

Blocking:

There are a lot of options to consider for this section. Here are a few options:

Number of years as a smoker
Type of smoker / quantity of cigarettes used within a time period
Age of the smoker
Rural or urban lifestyle
Reason for smoking
Reason for quitting
Have they tried previous smoking cessation programs (and have failed)

Factorial Experimental Design:

Factor 1: Use of pharmaceuticals: drug or no drug

Factor 2: Group therapy : therapy or no therapy

This experiment would then be a 2 X 2.

For this study it may be considered unethical to provide no treatment to individuals that wish to stop smoking. In this instance, the research team could elect to run an incomplete factorial in which there would not be a placebo group (no form of therapy).

References (does not include references for paper airplanes)

[1] D.P. Byar, A.M. Haerzberg, and W. Tan, "Incomplete factorial designs for randomized clinical trials," *Statistics in Medicine*, vol. 12, 1629 – 1641, 1993.

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Enhancing Experimental Design: Minimizing Confounding Variables

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Experimental design needs to…..

1. Promote the ability to provide a valid assessment of the interaction between the independent and dependent variables
2. Minimizes the possibility of alternative explanations

Both of these goals are linked to the presence of confounding variables

Alternative Explanations: Confounding Variables

- Confounding variables
  - Can change when the independent variable changes
  - Have an impact on the dependent variable (output of the experiment)

Example: When I am walking my dog she goes “crazy” when she sees another dog. To test this observation I setup an experiment to walk the dog 4 times in the presence of a dog and 4 times in the absence of a dog. Results: dog only barked when another dog was present.

What are the confounding variables?

Controlling for Confounding Variables

- Think of a confounding variable as “noise” that can camouflage the “signal” of the independent variable(s)
- The goal in controlling for confounding variables is to separate and minimize the “noise” from the “signal” to effectively analyze data
- Options to address confounding effects in experimental design
  - Enhance the independent variable signal: factorial design
  - Reduce the confounding variable noise: blocking

Enhance the Signal: Complete Factorial Design

- An efficient experimental design process that allows for the evaluation of more than one independent variable
- Enables for the evaluation of interactions between factors

In factorial design the following terminology is used:

- Factor a major independent variable
- Level is a subdivision of a factor

Example: If time is a factor then 1 hour, 2 hours, 3 hours would be the levels.

Factorials are expressed as

#levels for factor1 X # levels for factor 2 X # levels for factor N

Complete Factorial Design

Determining an effective dosage of hormone therapy to treat age related osteoporosis.

Factors	Levels
Dosage level	10 mg, 100 mg, 1000 mg
Age	40 – 50, 50 – 60, 60 - 70

There are two factors = dosage level and age

For each factor there are three levels

3 X 3 = 9 experimental runs

10, 100, 1000 mg

40-50, 50-60, 60-70

Example of a Complete Factorial Experiment: Weight loss trial with three factors

Diet (calories/day)	Exercise (time/day)	Sleep (time/day)	Experiment run
1500 calories	30 minutes	>8 hours	1
1500 calories	1 hour	>8 hours	2
1500 calories	30 minutes	<8 hours	3
1500 calories	1 hour	<8 hours	4
1200 calories	30 minutes	>8 hours	5
1200 calories	1 hour	>8 hours	6
1200 calories	30 minutes	<8 hours	7
1200 calories	1 hour	<8 hours	8

What is the Factorial Design?

In an experiment to determine the effect of antimicrobial solutions on shopping cart handles there are three different antimicrobial agents that will be tested plus a control. The effectiveness of each agent and control will be evaluated on the plastic and metal surfaces present on the handle of a shopping cart. Effectiveness of testing agents will be evaluated at 1, 10, 20, and 30 minutes post application.

How many factors?

How many levels for each factor?

What is the factorial design?

Other Forms of Factorial Design

- Fractional Factorial Design
  - Involves running only a subset of experimental runs
    - Often used when the number of experimental runs is unrealistic
    - Saves on money and resources
- Incomplete Factorial Design
  - A subset of a complete factorial design
  - Designs may not be balanced
  - Common in clinical studies

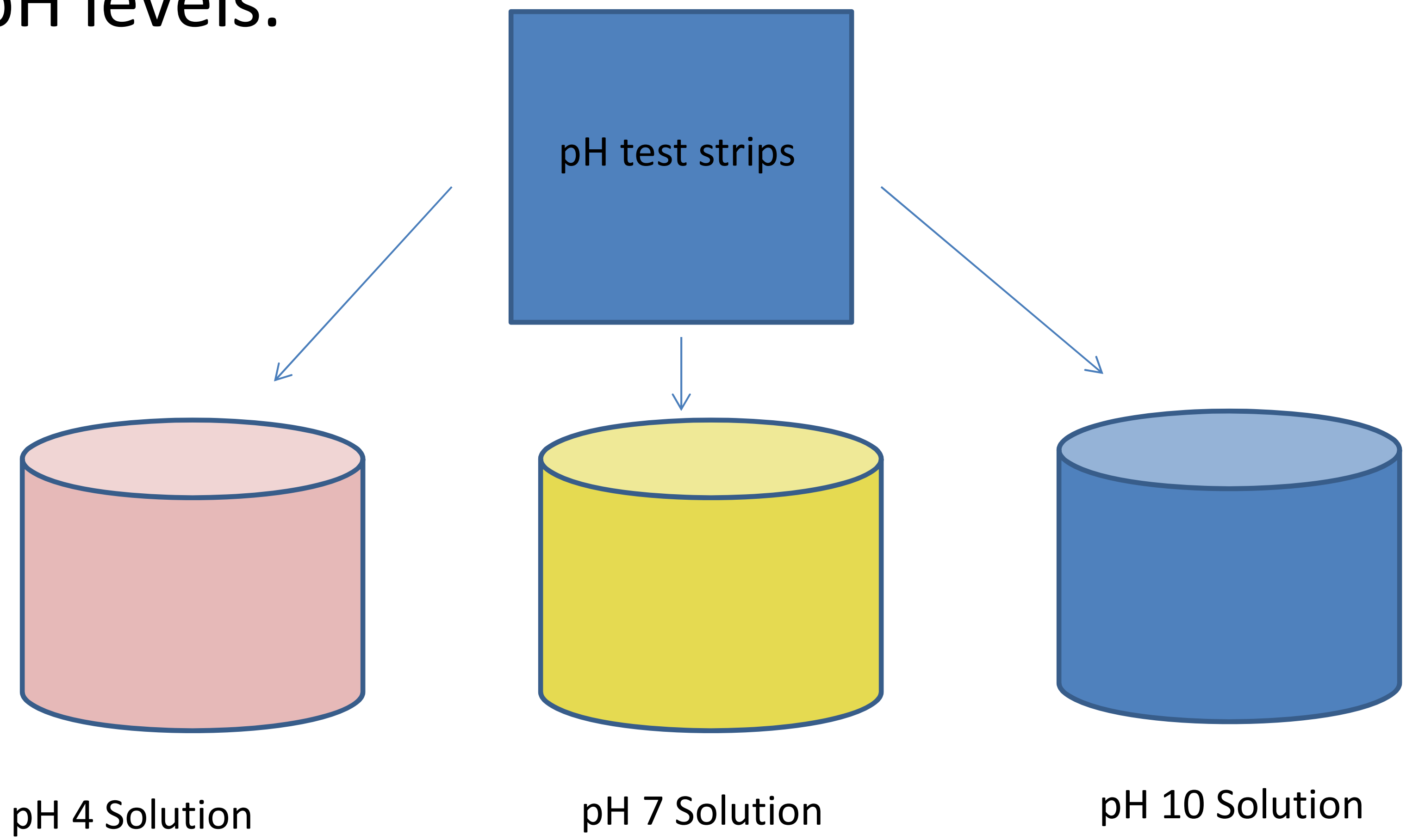
Decrease the noise: Blocking

- **Completely randomized block design (CRD)**: random assignment to groups
  - Used for homogenous populations
  - Running a 1 factor experiment (with no confounding variables) with multiple levels
  - Usually determined by a computer programs

- **Randomized block design (RBD)**: divide subjects into homogenous subgroups called blocks.
  - Blocking factors are sources of variability within a population. In clinical trials considerations for gender, race, age, disease state may be considered as blocks.

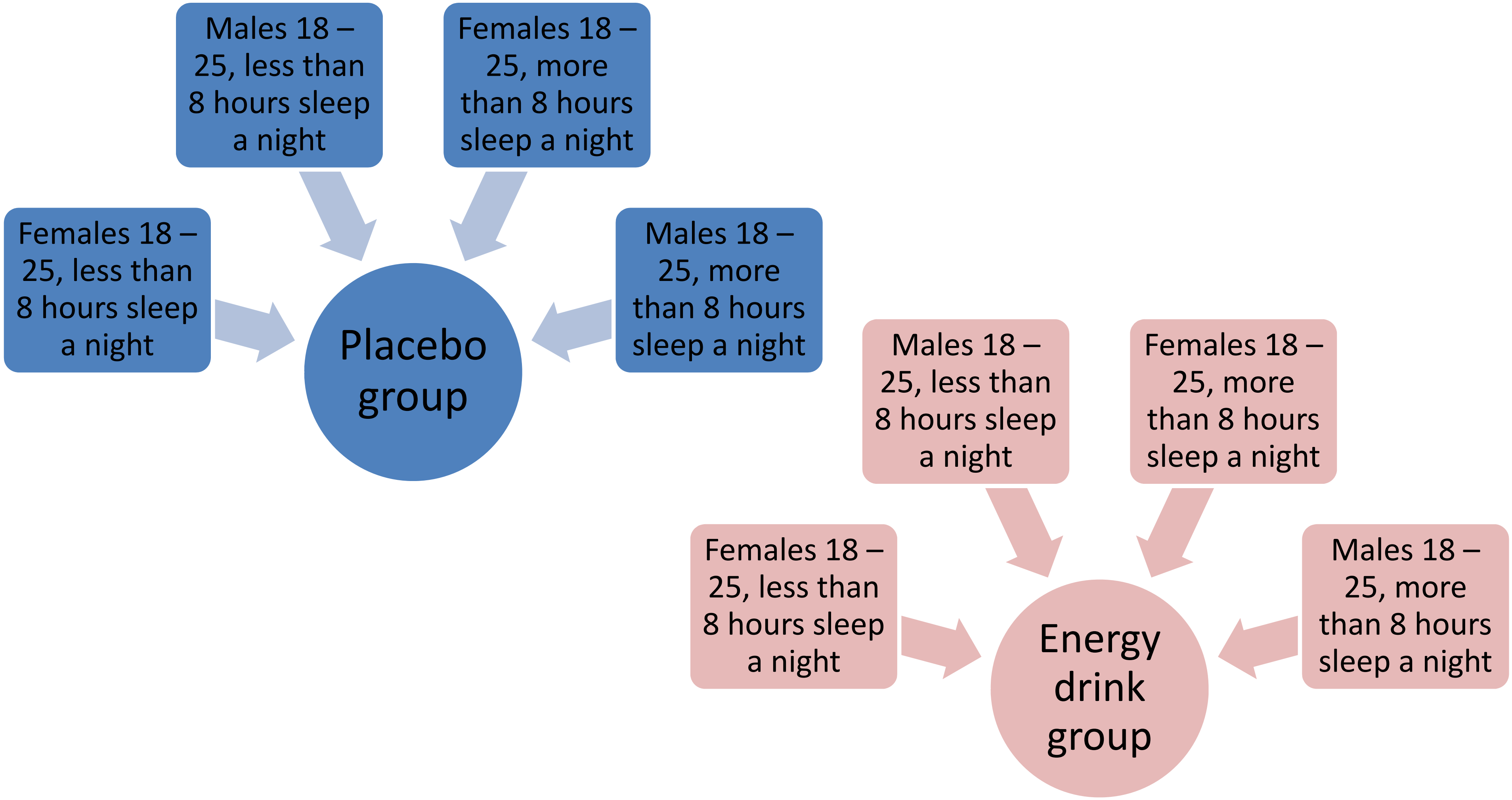
Example of Completely Randomized Block Design (CRD)

- To test pH strip on ability to detect different pH levels.



Example of a Randomized Block Design (RBD)

Determine if a new energy drink helps to maintain high energy levels?



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