



# LP5 TEACHING PLAN

## Experimental Design and Reproducibility



## LEARNING PLAN OVERVIEW

This learning plan will introduce students to the scientific method and will examine characteristics of well-designed and well-reported experiments. The module will also explore the reproducibility crisis in science.

## ESTIMATED TOTAL TIME

140 minutes

## WHAT STUDENTS WILL LEARN

**Competency:** Critique elements of experimental design and reporting for reproducibility and validity

### Learning Objectives:

- Explain the steps of the scientific method
- Describe the characteristics of quality scientific research
- Explain current threats to quality scientific research including faulty experimental design, lack of reproducibility, and pressure to publish
- Describe experimental design factors that impact experiment reproducibility
- Identify reporting factors that impact experimental reproducibility
- Identify steps researchers take to increase the validity of results
- Identify strategies for applying the 3Rs to improve research design and validity

### Assessment:

Case Study: Forced Swim Test

- Identify the control, independent, and dependent variables
- List steps taken to increase the validity of results
- Correlate the study to the 10 essential ARRIVE guidelines
- Identify potential limitations of the experimental design
- Consider data from subsequent human study applications and draw conclusions about the effectiveness of the animal study

### Linked External Standards:

CCSS- ELA

- RST.11-12.7 Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem
- RST.11-12.8 Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information
- RST.11-12.9 Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible

- W.9-10.9 Draw evidence from literary or informational texts to support analysis, reflection, and research

CCSS - Math

- HSN-Q.A.1 Use units as a way to understand problems and to guide the solution of multi-step problems; choose and interpret units consistently in formulas; choose and interpret the scale and the origin in graphs and data displays

## TEACHING PLAN

#	Learning Activities	Teaching Notes	Materials and Supplies
1	<p><b>LEARNING PLAN OVERVIEW</b> Review information detailed in the Student Learning Plan.</p>	<p>TIME: 5 minutes ACTIVITY NOTES: Provide a brief introduction to the learning plan.</p>	Student Learning Plan
2	<p><b>MOTIVATION ACTIVITY</b> Watch a short video from the science journal <i>Nature</i> to learn more about the reproducibility crisis in science.</p> <p>Briefly discuss the term reproducibility as it relates to scientific research. Brainstorm consequences of irreproducible research.</p>	<p>TIME: 5 minutes ACTIVITY NOTES: Have students watch a short video on the reproducibility crisis.</p> <p>After watching the video, discuss with students some of the consequences of irreproducible research:</p> <ul style="list-style-type: none"> <li>• Scientists assume that what they read is true and accurate in scientific publications and build on that information in their own research.</li> <li>• If the research a scientist builds their own work on is not reproducible, then they are wasting time, money, and possibly animal lives, on research that has a rocky foundation.</li> </ul>	<p>Video: Is there a reproducibility crisis in science? (2:03)</p> <p><a href="https://www.youtube.com/watch?v=j7K3s_vi_1Y">https://www.youtube.com/watch?v=j7K3s_vi_1Y</a></p>
3	<p><b>COMPREHENSION ACTIVITY</b> View a presentation on "The Scientific Method and Experimental Design."</p>	<p>TIME: 20 minutes ACTIVITY NOTES: Present "The Scientific Method and Experimental Design" slides provided.</p>	LP5_1_TheScientificMethodAndExperimentalDesign
4	<p><b>PRACTICE ACTIVITY</b> Use information from "The Scientific Method and Experimental Design Presentation" to complete "The Scientific Method and Experimental Design" worksheet.</p>	<p>TIME: 15 minutes ACTIVITY NOTES: Students may complete this on their own, with a partner, or in a small group.</p>	<p>LP5_2_TheScientificMethodAndExperimentalDesign_Worksheet</p> <p>LP5_2_TheScientificMethodAndExperimentalDesign_AnswerKey</p>
5	<p><b>COMPREHENSION ACTIVITY</b> Read an article to learn more about the reproducibility crisis in science.</p>	<p>TIME: 15 minutes ACTIVITY NOTES: Provide the article to the students. This scientific article is 3 pages. Questions on this article are provided in "The Reproducibility Crisis in Science" worksheet. The article and associated worksheet can be completed individually or in small groups.</p>	Access journal article here: <a href="https://www.nature.com/articles/533452a">https://www.nature.com/articles/533452a</a>

#	Learning Activities	Teaching Notes	Materials and Supplies
6	<p><b>PRACTICE ACTIVITY</b> Use the article information to complete “The Reproducibility Crisis in Science” worksheet.</p>	<p>Time: 15 min</p> <p><b>ACTIVITY NOTES:</b> Students may complete this on their own, with a partner, or in a small group. This worksheet can be provided to students along with the article.</p>	<p>LP5_3_TheReproducibilityCrisisInScience_Worksheet</p> <p>LP5_3_TheReproducibilityCrisisInScience_AnswerKey</p>
7	<p><b>COMPREHENSION ACTIVITY</b> View a presentation on “Considerations for Well-Designed Animal Experiments.”</p>	<p>TIME: 15 minutes</p> <p><b>ACTIVITY NOTES:</b> Present the “Considerations for Well-Designed Animal Experiments” slides provided.</p>	<p>LP5_4_ConsiderationsForWell-DesignedAnimalExperiments</p>
8	<p><b>PRACTICE ACTIVITY</b> Read the information on the “ARRIVE Guidelines Worksheet.” ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines are a checklist of information that researchers should include when publishing animal research. Investigate assigned guidelines and share findings as directed by your instructor.</p>	<p>TIME: 25 minutes</p> <p><b>ACTIVITY NOTES:</b> Provide students with the “ARRIVE Guidelines Worksheet.” Have them work in small groups to learn more about the ARRIVE Essential 10 items that should be included in a publication involving animal research.</p> <p>Assign each group 1 or 2 guidelines to investigate further. Inform students they will be sharing the information they learn about their assigned guidelines with the class.</p> <p>Reconvene the class to review the basics of the Essential 10 ARRIVE guidelines.</p>	<p>LP5_5_ARRIVE_Guidelines_Worksheet</p> <p>A PDF summary of the ARRIVE guidelines can be found here as a resource for teachers, students, or both:</p> <p><a href="https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20guidelines%202.0%20-%20English.pdf">https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20guidelines%202.0%20-%20English.pdf</a></p>
9	<p><b>APPLICATION ACTIVITY</b> Watch the Forced Swim Test video. Complete the “Case Study: Forced Swim Test” worksheet to examine whether the best practices of experimental design were applied in this case study. Refer to the Essential 10 ARRIVE guidelines document or website as needed.</p>	<p>TIME: 25 minutes</p> <p><b>ACTIVITY NOTES:</b> Play the Forced Swim Test video (30 sec) to provide a visual example related to the case study. Note that these experiments last longer than 30 seconds.</p> <p>In this case study, students are presented with a scenario where they have been asked to examine the methods for an experiment involving the Forced Swim Test.</p> <p>Have students complete this individually or in small groups.</p> <p>Discuss the answers with the class.</p>	<p>Forced swim test video: <a href="https://www.youtube.com/watch?v=WluxOBmo6s">https://www.youtube.com/watch?v=WluxOBmo6s</a></p> <p>LP5_6_CaseStudy_ForcedSwimTest_Worksheet</p> <p>LP5_6_CaseStudy_ForcedSwimTest_Answer Key</p>

# The Scientific Method and Experimental Design Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

## Part A: The Scientific Method

Use this worksheet to apply the steps of the scientific method to an everyday problem: your cell phone is not able to charge.

- 1. Make an observation.** Imagine that you plug your cell phone into its charger. You come back an hour later only to discover that your cell phone didn't charge. What is your observation?
- 2. Ask a question.** What question can you ask about the observation that you made?
- 3. Form a hypothesis.** A hypothesis is a testable explanation based on evidence. What is your hypothesis about why your phone didn't charge?
- 4. Make a prediction based on the hypothesis.** Frame the prediction as an "if...then" statement.
- 5. Test with an experiment.** What experiment would you perform to test your hypothesis?
- 6. Use the results to make new hypotheses or predictions.** Identify two different outcomes that could occur from your experiment. Determine whether the outcomes support your hypothesis.



# The Scientific Method and Experimental Design Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

## Part B: Experimental Design

### Questions on Experimental Design

1. A student is working in a cancer biology lab and is interested in seeing how Drug A affects cancer cell growth.  
How should the control group of cells be treated in this experiment?  
How should the experimental group of cells be treated in this experiment?
2. The student distributes an equal number of cancer cells to two petri dishes. She gives a dose of Drug A to the first dish but does not give any of the drug to the other dish. She lets the cells grow for two days and then counts them.  
What are the independent and dependent variables in this experiment?
3. She observes that the cells given Drug A have not grown as quickly as those without the drug and wonders if she may have discovered a potential treatment for cancer.  
What can the student do to increase the validity of the results she obtained in this experiment?



# The Scientific Method and Experimental Design Worksheet

## Answer Key

### Part A: The Scientific Method

Use this worksheet to apply the steps of the scientific method to an everyday problem: your cell phone is not able to charge.

- 1. Make an observation.** Imagine that you plug your cell phone into its charger. You come back an hour later only to discover that your cell phone didn't charge. What is your observation? **The cell phone did not charge.**
- 2. Ask a question.** What question can you ask about the observation that you made? **Why didn't the cell phone charge when plugged into the charger?**
- 3. Form a hypothesis.** A hypothesis is a testable explanation based on evidence. What is your hypothesis about why your phone didn't charge? **Answers will vary. Example hypotheses: The charger is broken or the outlet isn't working.**
- 4. Make a prediction based on the hypothesis.** Frame the prediction as an "if...then" statement. **Answers will vary based on the hypothesis selected. Example: If I use a different phone charger, then my cell phone will charge.**
- 5. Test with an experiment.** What experiment would you perform to test your hypothesis? **Answers will vary based on the hypothesis selected. Example: Use a different phone charger to charge your phone.**
- 6. Use the results to make new hypotheses or predictions.** Identify two different outcomes that could occur from your experiment. Determine whether the outcomes support your hypothesis. **Answers will vary based on the hypothesis selected. Example: If the phone charger successfully charges your phone, then your hypothesis is supported. If your phone doesn't charge with the new charger, then your hypothesis is not supported. Consider a new hypothesis.**



# The Scientific Method and Experimental Design Worksheet

## Answer Key

### Part B: Experimental Design

#### Questions on Experimental Design

1. A student is working in a cancer biology lab and is interested in seeing how Drug A affects cancer cell growth.  
How should the control group of cells be treated in this experiment?  
**It should not be treated with Drug A.**

How should the experimental group of cells be treated in this experiment?  
**It should be treated with Drug A.**
2. The student distributes an equal number of cancer cells to two petri dishes. She gives a dose of Drug A to the first dish but does not give any of the drug to the other dish. She lets the cells grow for two days and then counts them.  
What are the independent and dependent variables in this experiment?  
**The independent variable is the amount of Drug A given.**  
**The dependent variable is cell growth.**  
**Cell growth depends on the amount of Drug A given.**
3. She observes that the cells given Drug A have not grown as quickly as those without the drug and wonders if she may have discovered a potential treatment for cancer.  
What can the student do to increase the validity of the results she obtained in this experiment?  
**Repeat the experiment several times. Conduct statistical tests to see if the results obtained are significant.**



# A Survey about the Reproducibility Crisis in Science

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

In 2016, the science journal *Nature* published an article entitled “1,500 scientists lift the lid on reproducibility,” which highlighted the responses that researchers provided to a survey about reproducibility in their specific fields and in science in general. Access the journal article at <https://www.nature.com/articles/533452a>.

Refer to the article and answer the following questions.

1. What percentage of researchers polled believe there is a reproducibility crisis in science?
2. What are the top three fields of research in which scientists struggled to reproduce the results of others in their field?
3. According to the article, what are the top five factors that always or often contribute to irreproducible research?
4. What percentage of respondents indicated that their lab did not have established procedures for reproducibility?
5. In labs that have established procedures for reproducibility, what are some of the common practices they use to improve reproducibility?
6. What are the top three approaches that researchers feel could improve the reproducibility crisis in science?



# A Survey about the Reproducibility Crisis in Science

## Answer Key

In 2016, the science journal *Nature* published an article entitled “1,500 scientists lift the lid on reproducibility,” which highlighted the responses that researchers provided to a survey about reproducibility in their specific fields and in science in general.

Refer to the article and answer the following questions.

1. What percentage of researchers polled believe there is a reproducibility crisis in science?  
**Slight crisis (38%) + Significant crisis (52%) = 90% who believe there is a crisis**
2. What are the top three fields of research in which scientists struggled to reproduce the results of others in their field? **Chemistry, biology, and physics and engineering**
3. According to the article, what are the top five factors that always or often contribute to irreproducible research? **Selective reporting, pressure to publish, low statistical power or poor analysis, not replicated enough in the original lab, insufficient oversight/mentoring**
4. What percentage of respondents indicated that their lab did not have established procedures for reproducibility? **34%**
5. In labs that have established procedures for reproducibility, what are some of the common practices they use to improve reproducibility? **Repeating experiments themselves or asking others in the lab to repeat the work are among the most common practices. Making the effort to improve documentation of experimental methods and results and standardization of experimental methods are also helpful for improving reproducibility. Submitting hypothesis and data analysis plans for third party analysis prior to the experiment to prevent data cherry picking.**
6. What are the top three approaches that researchers feel could improve the reproducibility crisis in science? **More robust experimental design, better statistics, and better mentorship**



# ARRIVE Guidelines Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

Researchers working with animal models can consult a set of guidelines before they publish their work to improve the reporting of research involving animals. They are called the ARRIVE guidelines, which stands for Animal Research: Reporting of In Vivo Experiments. You can learn more about them at [arriveguidelines.org](http://arriveguidelines.org).

There are 10 guidelines considered essential and the basic minimum that researchers working with animal models should include in any publication describing animal research. Failure to include this basic information makes it impossible for readers of the paper to assess how reliable the research findings are.

These 10 essential guidelines are:

1. Study design
2. Sample size
3. Inclusion and exclusion criteria
4. Randomization
5. Blinding
6. Outcome measures
7. Statistical methods
8. Experimental animals
9. Experimental procedures
10. Results

Your instructor will break the class up into small groups and assign each group 1-2 guidelines to learn about in more detail. Visit the <https://arriveguidelines.org/arrive-guidelines> website to learn more about the specific guidelines your instructor has assigned to you.

Review both the explanation of the guideline as well as examples provided. Prepare to present a summary of your assigned guideline(s) to the class, as well as an explanation of how inclusion of this information can strengthen the publication of experiments involving animals.



# Case Study: Forced Swim Test Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

The Forced Swim Test was developed in the 1970s to examine the effectiveness of antidepressant treatments in rodents. In the Forced Swim Test, rodents are placed in a pool of water, usually for 5-15 minutes. During this time, the animals alternate between swimming and floating. Researchers have attributed the more passive floating posture with “despair” and depressive-like behaviors. For the purposes of this test, researchers compare how long the animal swims versus floats when given drugs that are being tested to treat depression.

We will examine the concepts of good experimental design using the Forced Swim Test as a case study.

Imagine that a group of researchers used the Forced Swim Test to examine the efficacy of a new drug as an antidepressant. When testing this compound in the lab, they obtained promising results and wanted to write up their findings for publication in a scientific journal.

Review the methods that they included in their scientific paper and answer the questions that follow.

- Sixteen-week-old C57Bl/6J male and female mice were used in these experiments. They were housed individually in standard shoebox cages at 23°C and 45-55% humidity. Food and water were available to animals throughout the duration of the experiment.
- One week prior to the Forced Swim Test, animals were injected daily with 1 mg/kg or 5 mg/kg of the drug or an equal volume of saline solution as a control. Mice were randomly allocated into treatment groups for these studies.
- Animals were subjected to the Forced Swim Test for six minutes. Depression-like behavior was measured by an increased duration of immobility in the Forced Swim Test. Experiments were repeated three times. A student t-test was performed to examine differences in immobility times between treated and untreated animals. P-values of 0.05 or less were considered significant.



# Case Study: Forced Swim Test Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

1. Does this experiment have a proper control? If yes, what is the control? If not, what should it be?
2. What are the independent and dependent variables in this experiment?
3. What, if any, steps did the researchers take to increase the validity of the results they obtained in this experiment?



# Case Study: Forced Swim Test Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

4. Consider the information that the researchers provided in the methods section of their scientific paper. Describe how the scientists failed or succeeded in addressing each of the 10 Essential ARRIVE guidelines.
1. Study design
  2. Sample size
  3. Inclusion and exclusion criteria
  4. Randomization
  5. Blinding
  6. Outcome measures
  7. Statistical methods
  8. Experimental animals
  9. Experimental procedures
  10. Results - Only the methods section of the paper was provided, so results were not included for you to analyze.



# Case Study: Forced Swim Test Worksheet

Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

Although efforts to include more detailed information in scientific publications may help with the reproducibility crisis, there are issues with animal experiments that extend beyond study design and reporting. They include issues such as translating data across species.

For example, a paper came out in 2021 that analyzed whether drugs tested in the Forced Swim Test by large pharmaceutical companies had antidepressant effects in people.

The authors of the paper conducted a retrospective analysis, focusing on available data from the 15 pharmaceutical companies with the largest generated revenue, to see whether the Forced Swim Test correctly informed human clinical trials and predicted human clinical efficacy.

Of the 109 drugs they identified that were tested for treating antidepressant behavior using the Forced Swim Test, only 28% were further investigated for effects on depression in humans. Of those compounds, only 23% were predictive of the outcome in humans. And none of those compounds have been marketed to treat human depression.

The authors concluded that the Forced Swim Test is not a reliable screening tool for antidepressant drugs by pharmaceutical companies and recommended that its use be suspended while more data are collected. They further recommended that this controversial test be discontinued if the collection of additional data found similar results.

5. Consider the way that depression is analyzed in the Forced Swim Test. Can you identify any limitations with this approach?
6. Do you agree or disagree with the author's conclusion that the Forced Swim Test be discontinued? Why or why not?



# Case Study: Forced Swim Test Worksheet

## Answer Key

1. Does this experiment have a proper control? If yes, what is the control? If not, what should it be?  
**The mice given the saline solution serve as a control in this experiment, as they were not given the drug being tested.**
2. What are the independent and dependent variables in this experiment?  
**The independent variable is the amount of the drug.**  
**The dependent variable is amount of time that the animals are immobile in the Forced Swim Test.**
3. What, if any, steps did the researchers take to increase the validity of the results they obtained in this experiment? **They repeated the experiment three times and conducted statistical analyses to look for significant differences among the treated and untreated animals. They also took steps to minimize bias by randomly assigning animals to control or experimental groups.**



# Case Study: Forced Swim Test Worksheet

## Answer Key

4. Consider the information that the researchers provided in the methods section of their scientific paper. Describe how the scientists failed or succeeded in addressing each of the 10 Essential ARRIVE guidelines.
  1. Study design  
**Information about the study design, including the groups being compared and control groups, were mentioned.**
  2. Sample size  
**The number of animals allocated to each group and the total number of animals used was not included in the methods.**
  3. Inclusion and exclusion criteria  
**Criteria used for including or excluding animals was not explained. Researchers also did not mention if criteria were in place to include or exclude animals.**
  4. Randomization  
**The researchers indicated that animals were randomly assigned to treatment groups.**
  5. Blinding  
**The researchers did not mention whether they were blinded during any point of the experiment.**
  6. Outcome measures  
**The outcome of “increased duration of immobility” was mentioned in the methods.**
  7. Statistical methods  
**The researchers mention that a t-test was performed as part of the data analysis for the experiment.**
  8. Experimental animals  
**The researchers provided species-appropriate details of the animals used in the experiment, including species, strain, sex, and age.**
  9. Experimental procedures  
**Very few details of what was done, how it was done, and what was used were provided. More information would be needed here for another researcher to be able to replicate this experiment.**
  10. Results  
**We only provided the methods section of the paper to you, so results were not included for you to analyze.**



# Case Study: Forced Swim Test Worksheet

## Answer Key

Although efforts to include more detailed information in scientific publications may help with the reproducibility crisis, there are issues with animal experiments that extend beyond study design and reporting. They include issues such as translating data across species.

For example, a paper came out in 2021 that analyzed whether drugs tested in the Forced Swim Test by large pharmaceutical companies had antidepressant effects in people.

The authors of the paper conducted a retrospective analysis, focusing on available data from the 15 pharmaceutical companies with the largest generated revenue, to see whether the Forced Swim Test correctly informed human clinical trials and predicted human clinical efficacy.

Of the 109 drugs they identified that were tested for treating antidepressant behavior using the Forced Swim Test, only 28% were further investigated for effects on depression in humans. Of those compounds, only 23% were predictive of the outcome in humans. And none of those compounds have been marketed to treat human depression.

The authors concluded that the Forced Swim Test is not a reliable screening tool for antidepressant drugs by pharmaceutical companies and recommended that its use be suspended while more data are collected. They further recommended that this controversial test be discontinued if the collection of additional data found similar results.

5. Consider the way that depression is analyzed in the Forced Swim Test. Can you identify any limitations with this approach? **It does not mirror how we would examine the effectiveness of antidepressant treatments in people. Mice likely experience “despair” and “depression” very differently than people do. There may be other explanations for why the animal swims or floats during the test that may have to do more with the animals’ ability to cope with being in an inescapable situation. Some people have ethical concerns about performing this test on animals as well.**
6. Do you agree or disagree with the author’s conclusion that the Forced Swim Test be discontinued? Why or why not? **Answers will vary.**

