

“Doing Science” Workshops

2nd Workshop

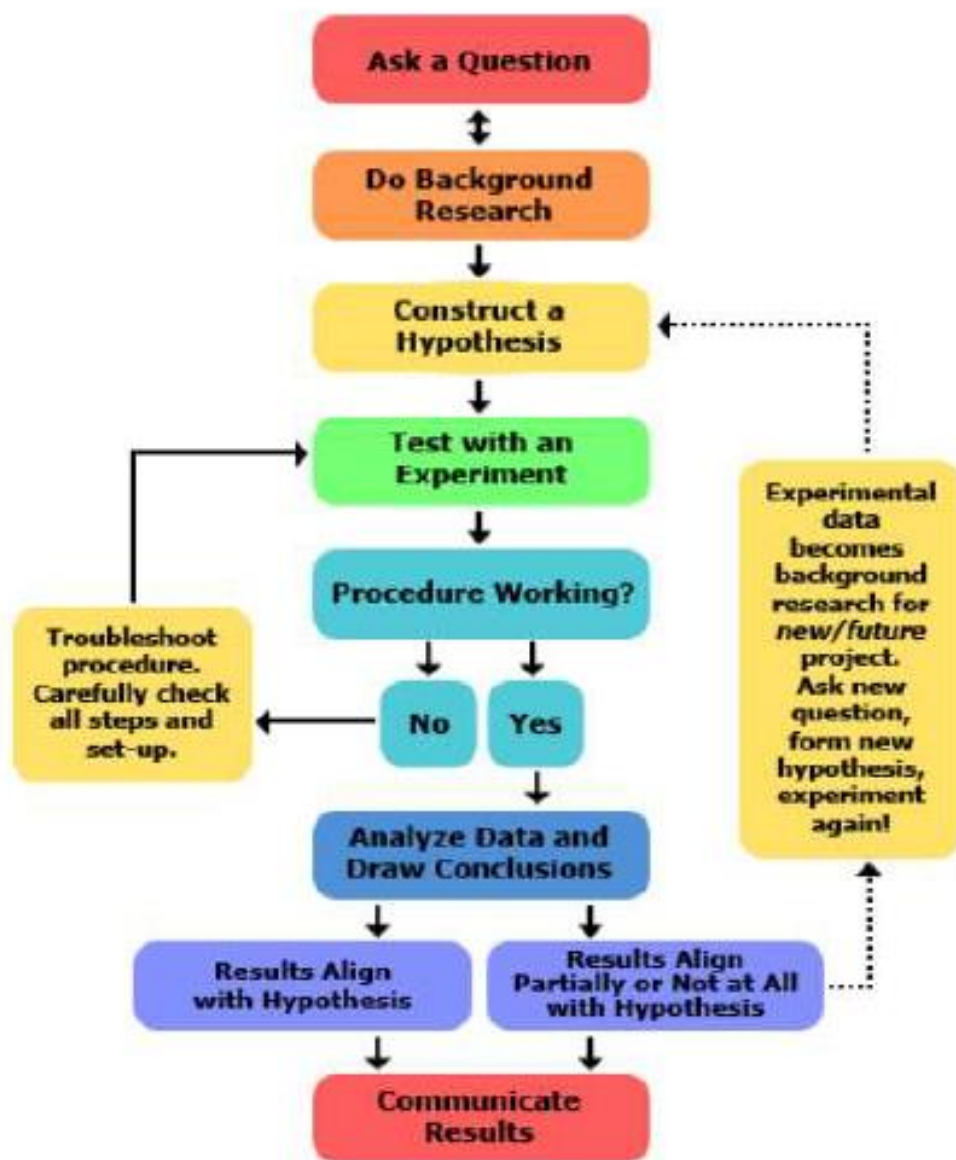
Dr. Felix E. Rivera-Mariani

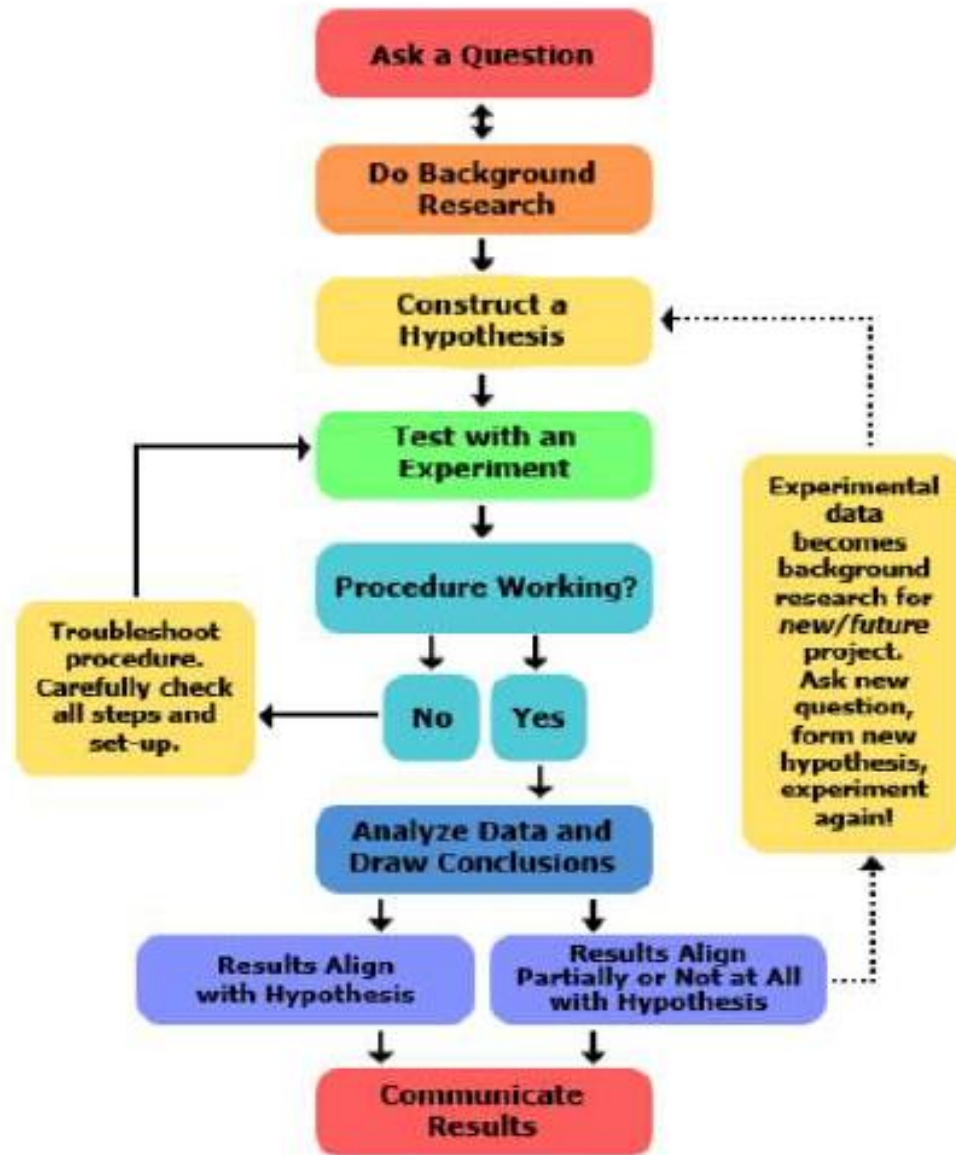
Learning Objectives in the Workshop Series

- Understand the different parts of the “real” scientific method
- Design workable goals through a scientific project
- Analyze the different thought processes towards a scientific goal
- Collect data in formats that are “easy”(or “less difficult”) to analyze
- Answer questions related to our data-collection process
- Value the importance of team-work in the scientific process
- Understand, elaborate, and communicate with a scientific mindset

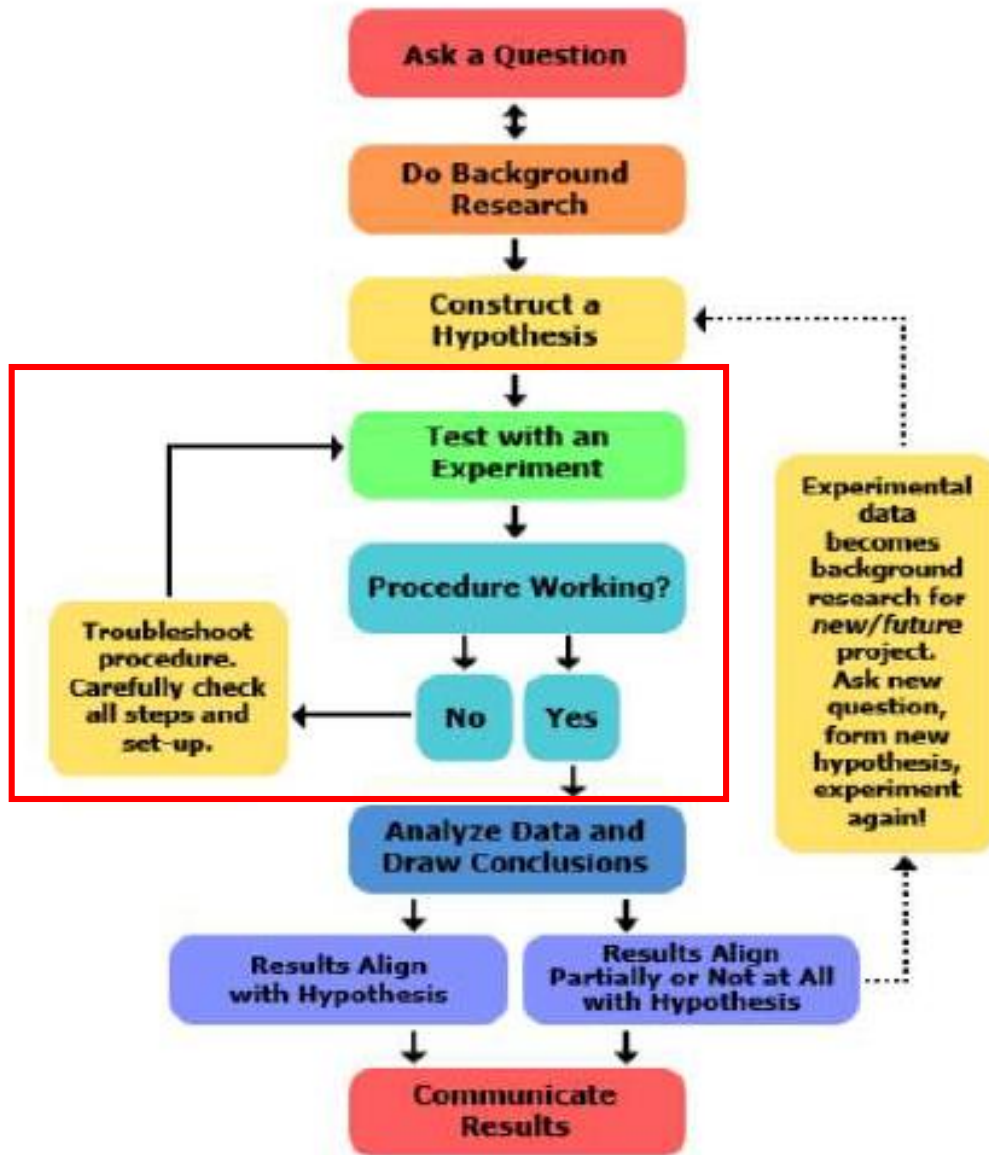
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At which steps of the scientific method epicycle will you spend the most time?



...designing and performing experiments and collecting data

Scientific method

1

- Ask a Question

2

- Do Background Research

3

- Construct a Hypothesis

Scientific method

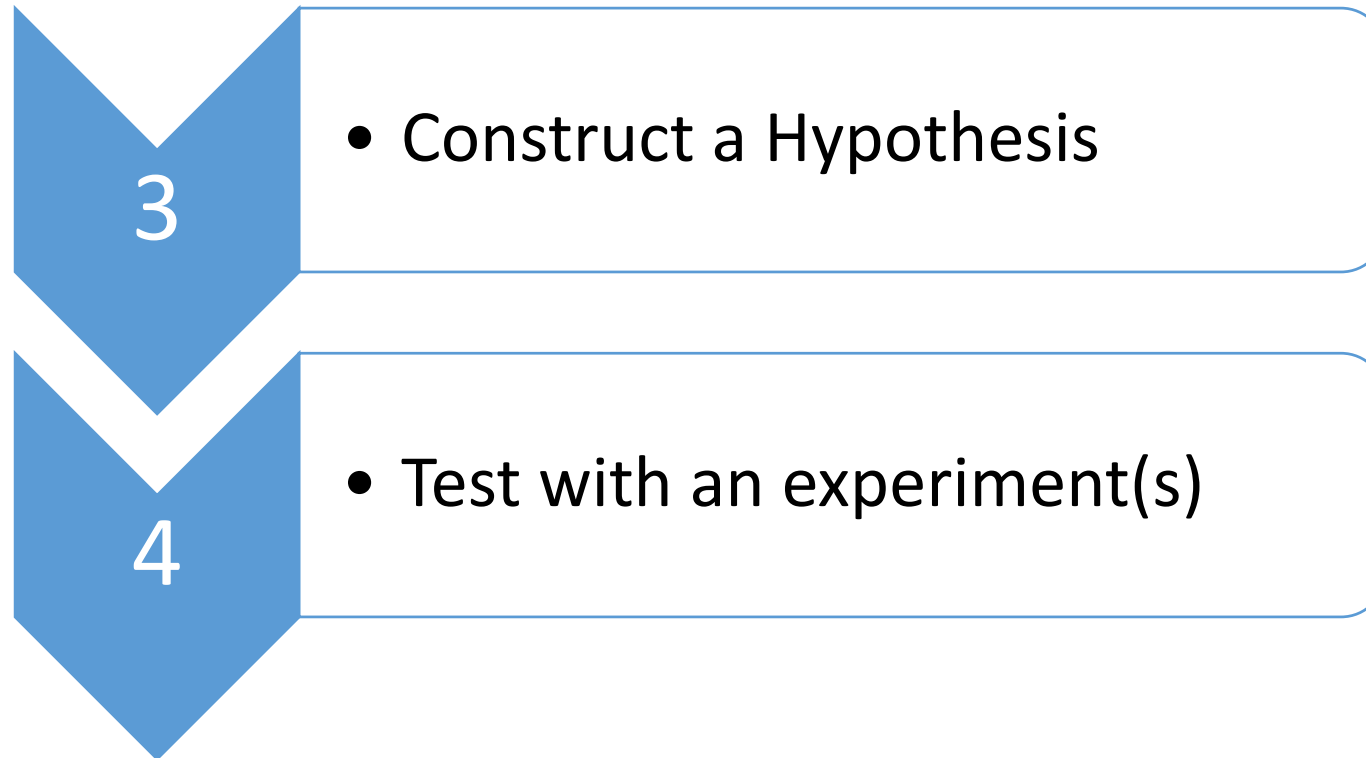
3

- Construct a Hypothesis

4

- Test with an experiment(s)

Scientific method



What we must consider when designing the experiments?

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

- That the experiments provide data to reject or fail to reject the hypotheses

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

- That the experiments provide data to reject or fail to reject the

hypotheses

- Null = H_0
- Alternative = H_A

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

-That the experiments provide data to reject or fail to reject the hypotheses

-We must have the equipment and materials

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

-That the experiments provide data to reject or fail to reject the hypotheses

-We must have the equipment and materials

-Must have knowledge with the proposed experiments

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

-That the experiments provide data to reject or fail to reject the hypotheses

-We must have the equipment and materials

-Must have knowledge with the proposed experiments

-Which are the variables?

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

-Be able to provide data to reject or fail to reject the hypotheses

-We must have the equipment and materials

-Must have knowledge with the proposed experiments

-Which are the variables?

-How to analyze the data

What we must consider when designing the experiments?

3

- Construct a Hypothesis

4

- Test with an experiment(s)

-That the experiments provide data to reject or fail to reject the hypotheses

-We must have the equipment and materials

-Must have knowledge with the proposed experiments

-Which are the variables?

-How to analyze the data

-Obtain permission

Immunodetection and quantification of airborne (1–3)- β -D-glucan-carrying particles with the halogen immunoassay

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Abstract

Fungal cell wall components, such as (1–3)- β -D-glucan, are known to be capable of activating the innate immune system and pose a respiratory health risk in different environments. Mass-based non-viable techniques commonly used for assessment of fungal exposures could be β -D-glucan-specific, but are limited to analysis of liquid extracts. The variable solubility of different β -D-glucans may underestimate β -D-glucan exposure and long sampling times required for mass-based methods make assessing short-term exposures difficult. In this study, we evaluated the utility of the halogen immunoassay (HIA), an immunoblotting technique previously used for allergens, to immunodetect and quantify β -D-glucan-carrying particles (BGCPs). The HIA was able to detect BGCPs without background staining when β -D-glucan standards and air samples collected at a poultry house during short sampling periods were evaluated. The image analysis protocol previously developed by our group for mouse allergen allowed simultaneous immunodetection and quantification of β -D-glucan-containing particles. Our results suggest that the HIA holds promise for quantifying β -D-glucan exposures. To our knowledge, this is the first time in which the HIA was used for non-allergenic compounds of microbial or fungal origins.

Identify the hypothesis, question or purpose?

Exercise with the questions previously discussed when designing experiments

Group homework

Comparison of the Interleukin-1 β -Inducing Potency of Allergenic Spores from Higher Fungi (Basidiomycetes) in a Cryopreserved Human Whole Blood System

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Identify the hypothesis, question or purpose?

Exercise with the questions previously discussed when designing experiments

Abstract is on the following page.

Key Words

Basidiospores · Human whole blood · Interleukin-1 β · Proinflammatory potency

Abstract

Background: Spores from basidiomycete fungi (basidiospores) are highly prevalent in the atmosphere of urban and rural settings. Studies have confirmed their potential to affect human health as allergens. Less is known about their potential to serve as stimuli of the innate immune system and induce proinflammatory reactions. **Methods:** In this study, we evaluated the proinflammatory potential of spores from 11 allergenic basidiomycete species (gilled: *Pleurotus ostreatus*, *Oudemansiella radicata*, *Armillaria tabescens*, *Coprinus micaceus*, *Pluteus cervinus*, and *Chlorophyllum molybdites*, and nongilled: *Pisolithus arhizus*, *Merulius tremellosus*, *Calvatia cyathiformis*, *Lycoperdon pyriforme*, and *Boletus bicolor*) based on their potency to induce the release of the proinflammatory cytokine interleukin (IL)-1 β in a cryopreserved human whole blood system. In addition, the roles of morphological features of the spores (surface area, shape, and pigmentation) were examined for their role in the IL-1 β -inducing potency of spores. Peripheral blood from healthy volunteers was collected, pooled, and cryopreserved. After

stimulating the cryopreserved pooled blood with 10⁶ to 10³ basidiospores/ml, the concentration of IL-1 β in culture supernatants was determined with ELISA. **Results:** Basidiospores manifested concentration-dependent IL-1 β -inducing potency, which was more marked among basidiospores from gilled basidiomycetes. At higher concentrations of basidiospores, the IL-1 β -inducing potency could be differentiated in the cryopreserved human whole blood system. Morphological features did not correlate with the IL-1 β -inducing potency of the basidiospores, suggesting that nonmorphological properties modulate the IL-1 β -inducing potency. **Conclusion:** Our data provide evidence of the proinflammatory potential of basidiospores, and the utility of cryopreserved human whole blood as a human-based in vitro system to study the immune reactivity of allergenic basidiospores.

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