Presentation on Scientific Methodology and N+N-1 Rule on Experimental Design (Harris-Stowe State Undergraduate Summer Workshop)

Scientific method and experimental design

BIO0403 (Harris-Stowe State University)

Undergraduate Summer Fellowship Lecture

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1

Outline

- Scientific Method
- Hypothesis
- Experiment
- Common causes of experimental failure
  - Blind trust in authority
    - Notebooks
  - Intolerable margin of error
    - Controls
    - N+(N-1) rule

2

What is Scientific Method?

- A body of techniques for acquiring new knowledge, or correcting/integrating previous knowledge;
- Based on empirical and measurable evidence subject to scientific principles of reasoning;
- Consisting in systematic observation, measurement, and experiment, and the formulation, testing, and modification of hypotheses *(Oxford English Dictionary)*

3

Elements of scientific method (progressive stepwise process)

1. Problem/Question
2. Observation (to gather some information about the question)
3. Formulate a Hypothesis (to answer the question)
4. Test the hypothesis by performing an experiment
5. Analyze the results (from data collected)
6. Interpret the data and draw conclusions (that serve as a starting point for new hypothesis)
7. Communicate the Results (Present to the audience, Publish the results)
8. Retest (frequently done by other scientists)

4

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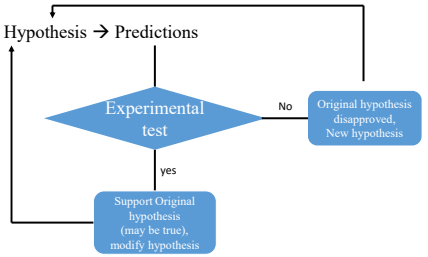
5

Formulate a hypothesis

- A proposed explanation for a phenomenon
- Based on previous observations that cannot satisfactorily be explained
- Testable
- Hypothesis ≠ Theory
  - Scientific Hypothesis: yet to be tested (may be true or false)
  - Scientific theory: already tested, generally accepted to be the accurate explanation behind an observation

6

Hypothesis → Predictions → experiment



7

Competing hypotheses

Example:

- DNA is a triple helix (X-ray result)
- DNA is a double helix
  - phosphate/sugar inside (unstable in water)
  - phosphate/sugar outside

8


April 25, 1953 NATURE 737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

9

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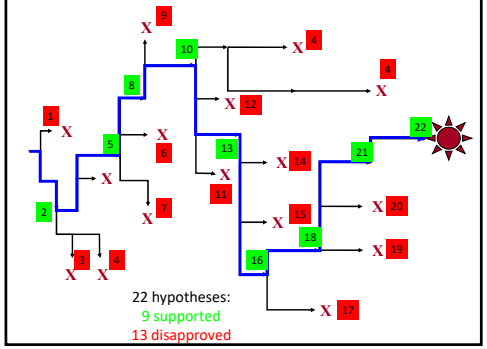
10

- **A successful experiment does not necessary generate data in support of the hypothesis.**
- **However, it must be reproducible and informative, because…**

11

- **Unproductive experimenters could not find the right way to solve the problem.**
- **They could not get the techniques to work reliably and predictably either.**

12



13

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14

Common causes of experimental failure

- ***Blind (excessive) trust in authorities***
- ***Intolerable margin of error***

— Key underlying causes of poor experimental design and failure

15

What or who are authorities (pre-existing knowledge)?

- **What — textbook, Scientific Journals, review articles, regular articles, thesis, meeting abstract, handbook, instrument manual, reagent data sheet, catalog, notebook ……**
- **Who—Nobel prize winner, thesis advisor, other professors, senior students, fellow students, friends, relatives, salesmen, politicians, reporters, ……**

16

Notebooks

Things to be recorded in a notebook should include everything related to this particular experiment:

- Date, purpose, background (reference, etc.), design (table, "N+(N-1)" rule), reagents/solutions (data sheet)
- protocol (flowchart, make remarks during the experiment)
- results (numbers/pictures, printouts, statistic analysis)
- discussion.

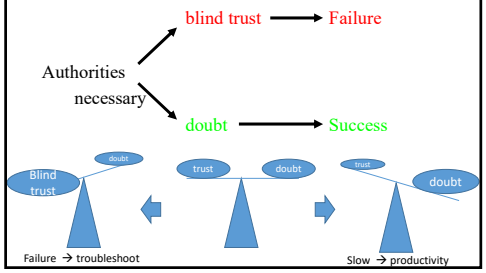
17

Examples of blind trust:

1. Special mouse strain, cell line,....
2. Antibody specificity,....
3. Pipetman setting
4. Water purity
5. Plasmid maps
6. pH meter readout.
7. CO2 level in the incubator.
8. Protocols from senior students
9. Ideas from meetings
10. ……

18

Each experiment is associated with a balance point between trust and necessary doubt



19

Seeing is believing, doing is knowing

- *To hear is to forget;*

  Everything you have heard from lectures you'll forget soon.

- *To see is to remember;*

  Whatever you have learned by watching others doing you'll remember for a while.

- *To do is to know!*

  Carrying out a challenging task with your own efforts makes it unforgettable.

20

Common causes of experimental failure

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21

Controls

- Always include controls in your experimental design.
- If positive controls do not work or not included, the negative results are absolutely meaningless.
- If negative controls do not work or not included, the positive results are absolutely meaningless.

22

How to optimize the conditions —*the "N + (N- 1)" rule*

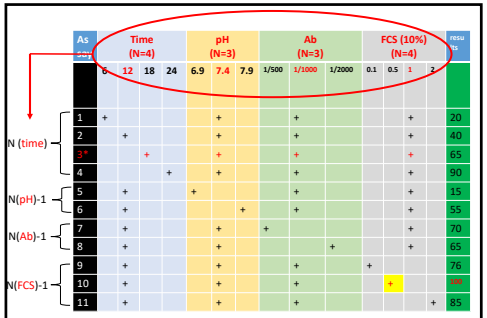
Independent variables	Range (margins)	N
Time (hr)	6, 12, 18, 24	4
pH	6.9, 7.4, 7.9	3
Ab(dilution)	1/500, 1/1000, 1/2000	3
FCS(%)	0.1, 0.5, 1, 2	4

The number of total experiments needed

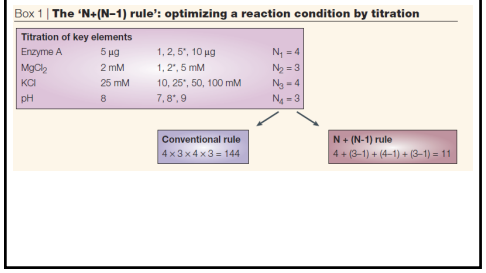
= 4 + (3-1) + (3-1) + (4-1) = 11

Conventional/thorough test would need 4X3x3x4= 144 assays

23



24



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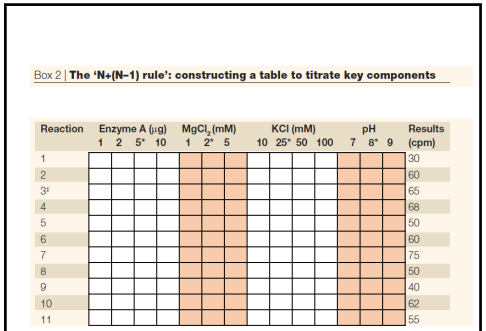
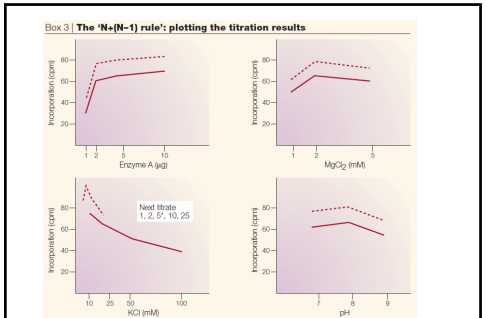


26



27

Box 2 | **The 'N+(N–1) rule': constructing a table to titrate key components**

Reaction	Enzyme A (µg)				$MgCl_2$ (mM)			KCl (mM)				pH			Results
	1	2	5*	10	1	2*	5	**1**	**2**	**5***	**10**	7	8*	9	(cpm)
1															
2															
3‡															
4															
5															
6															
7															
8															
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10															
11															

28

Elements of scientific method (progressive stepwise process)

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29

Thank you !

Questions?

30