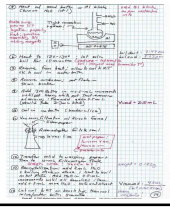
Maintaining a Lab Notebook

Your notebook is the proof of what you did AND what you/we were thinking.

Purpose of lab notebook

- Maintain records of your methods and results of experiment to pass on to others
- Legal document to prove patents and defend your data against accusations of fraud
- In your lab notebook, you may
  - Plan experiments
  - Build on your results
  - LEARN from your mistakes



Why does your PI care?

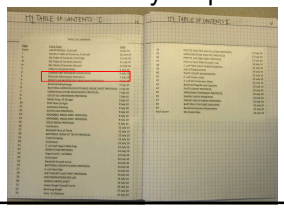
- Insurance policy for the PI and you to resolve claims of scientific fraud or misconduct
- Notebook is legal document of grant expenditure
- NIH can legally audit records that are relevant to any grant. They MUST be able to understand and verify that your calculations and procedures are as we published them.
- Keep for 5 yrs after grant ends or publication which ever is longest
- If a patent is applied for, the notebook is the legal document- Intellectual Property Law requires clear evidence of the date of invention- ideas can be patented too
- After you leave, your PI may need it for grants, papers, additional experiments (what and how you did things)

The basics

- Must be legible and provide enough information for others to replicate the study.
- Define any abbreviations that you use
- Most US PI's request it be in English
- Date everything!
- Keep where it can be easily accessed- PI may want to look at data on weekends!
- Notebooks do not leave the lab!

Table of contents

- Date, experiment title and date
- If doing multiple times, give basic info- worked, didn't work
- Keep a list of where your protocols are



Specifics

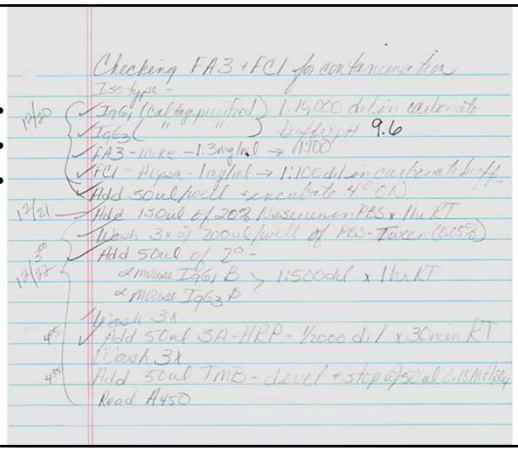
- Date and Title
- Intro- Justification of experiment- simple logic or reference-1 or 2 sentences
- Clearly worded hypotheses and goals
- Methods
- Summary/Conclusions observed and samples that were collected.
- Who helped with any aspect of the experiment to allow for acknowledgements?

Methods

- First time, lots more detail than repeats unless you make changes
- Show all calculations so that all numbers, concentrations, etc. are fully explained and interpretable by another researcher. Remember to include units
- Label all figures and calculations

The DETAILS!

- Reagents: source, product number, lot number, expiration date, how and where stored
- Solutions and how they were made (did you dilute from 10x stock or make from scratch?) Show calculations! What type of water was used? Include a recipe or put in protocols
- Cells used: type, source, passage number, growth medium
- Instruments: type, name, location, serial number
- Number and volume of washes
- Centrifuge speeds and duration of spins
- Heating rates and levels of agitation
- Time between and during steps
- Gel percentages



- Where are th data files are stored and what they are called? NOT on a USB drive!
- Record names of people providing assistance, techniques, statistical advice, equipment loans. Write this information down for "acknowledgements" sections.
- Using animals Keep the cage cards or at least all the information!

2nd time expts

- Refer to original protocol
- Record start/stop times of long incubations (1 hr or more)
- Record ANY changes- if you will repeat the changes, then rewrite the protocol COMPLETELY and refer to that protocol in subsequent expts. Put new protocol in Table of contents

Protocols

- Write out or attach the protocol the first time
- Reference the protocol page number on subsequent times
- Every time, write down times of beginning/ending long incubations, machine number
- Record ALL calculations as you do them. DO NOT TRANSFER info- it is NOT legal
- Detail all mistakes, problems with procedures, and lapses in data collection so that you can fully explain "odd" results at the end of your experiment.
- Record ANY changes to the protocol WHEN they occur
  - Dropped tube on floor, estimated volume left and added more
  - Reagent was 50 percent of the strength we originally thought.
  - Made 75% EtOH with 75% water not EtOH

Organize it!

Lab notebook is like a ship's log. It tells where you've been and where you are going and how you accomplished both

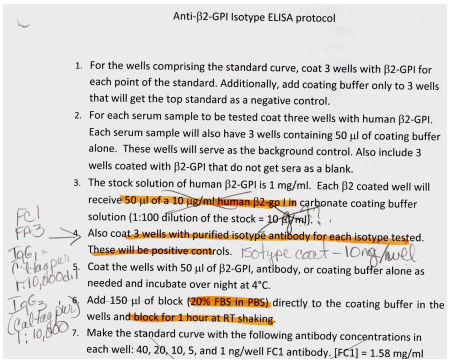
Do's and Don'ts

Do's

- Legible and orderly
- Ideas for next expts
- Enough for others to replicate
- Abbreviations- defined
- Details!!

Don'ts

- Modify- (line through)
- Rewriting scrap paper
- Skip pages
- Leave blank spaces
- Remove originals
- NO whiteout!



Anti-β2-GPI Isotype ELISA protocol

1. For the wells comprising the standard curve, coat 3 wells with β2-GPI for each point of the standard. Additionally, add coating buffer only to 3 wells that will get the top standard as a negative control.
2. For each serum sample to be tested coat three wells with human β2-GPI. Each serum sample will also have 3 wells containing 50 µl of coating buffer alone. These wells will serve as the background control. Also include 3 wells coated with β2-GPI that do not get sera as a blank.
3. The stock solution of human β2-GPI is 1 mg/ml. Each β2 coated well will receive 50 µl of a 10 µg/ml human β2-gp I in carbonate coating buffer solution (1:100 dilution of the stock = 10 µg/ml).
4. Also coat 3 wells with purified isotype antibody for each isotype tested. These will be positive controls.
5. Coat the wells with 50 µl of β2-GPI, antibody, or coating buffer alone as needed and incubate over night at 4°C.
6. Add 150 µl of block (20% FBS in PBS) directly to the coating buffer in the wells and block for 1 hour at RT shaking.
7. Make the standard curve with the following antibody concentrations in each well: 40, 20, 10, 5, and 1 ng/well FC1 antibody. [FC1] = 1.58 mg/ml

Who am I?

- Trisha Rettig
  - 4th year PhD student
    - Dr. Chapes (K-State)
    - trettig@ksu.edu
- Before that…
  - Worked 4.5 years in industry
  - 3rd party testing lab
  - GMP/GLP
  - Constant audits
    - FDA, ISO, EMA, clients

cGMPs

- Current Good Manufacturing Practices
  - Regulated by the FDA
  - Change to reflect "current"
  - Used for final drug products, medical devices, vaccines, etc.
- Cover lots of different areas
  - SOPs
  - Document handling
  - Data storage
  - Training
  - Documentation

What's the Point?

- Ensure a unalterable paper (data) trail of **what** happened
  - Audits (clients, FDA)
  - Legal action
- Official documents
- Used to ensure uniformity
  - Alterations resulted in deviations
- Can be in notebook form or worksheet form
  - Applies to test tubes!

Focus of cGMP Documentation

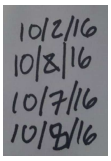
- Prevent alterations
  - Identify if changes were made
- Accurate record of what actually happened
  - Who did what?
  - How did they do it?
- Provide a record that can be checked by another party
  - QA
  - Clients, FDA

cGMP Documentation Rules

- Each person is assigned a set of initials to be used for documentation
  - TR, TAR
  - Multiple people cannot share initials
  - Signatures
  - Record of your initials and signature – maintained yearly
- Clearly documented
- Filled out **in real time**

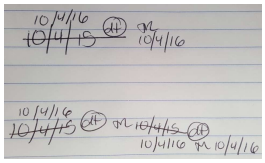
cGMP Documentation Rules

- Must be a permeant record
  - No white out, no sticky notes
- Fully explain calculations
  - 10 mg sample + 100 mL water = 1mg/mL. 1 mL of 1 mg/mL + 9 mL of water = 1:10 of 1 mg/mL = 0.1 mg/mL
- No write overs
- No scribbling out mistakes



cGMP Rules

- Single line out
- Error codes
  - tr, ee, dt, etc



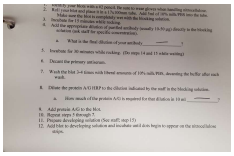
10/6/16 (dt) TR 10/6/16
~~10/7/16~~

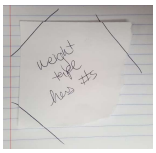
8 (ee) TR 10/6/16
~~5~~ mL of sample……

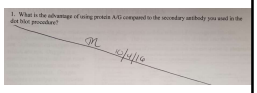
(tr) TR 10/6/16
Sample ID ~~05729~~
05838

Preventing Future Alterations

- Line out blank sections
  - Dashes, crosses, N/A
- Mark the corner of weigh tape
  - Permanent double sided tape







Conclusion

- cGMPs are designed to leave an accurate paper trail
- Prevent alterations
- Provide a document to be reviewed by others
  - QA
  - Clients, FDA, etc

Questions?