- Announcements
- Quiz (last one!)
- Pre-lab Lecture
  - DMMB assay
  - More about ELISA
  - qPCR analysis
  - Today in Lab (M3D6)

Announcements

- Lec 7: Atissa on proposal; Lec 8: special topics
- Next time: finish report + clean up; WAC survey
- Mod 3 research proposal
  - feedback on your wiki page today/tonight + collaborative DJ'ing
- Mod 3 report
  - no separate Methods section needed: *do* state any unique conditions along the way in Results section/captions
  - required analysis: viability (incl. stats), qPCR (incl. RNA amount and cell counts), ELISA (incl. standard curves)
  - optional/if relevant analysis: PG assay; general bead, cell, and media appearance on D2, D3, and/or D4

DMMB assay

- Measure GAGs with cationic dye
- Absorbance shift due to complex A525 ↓
  - fades quickly! (pipet upstairs)
  - at low pH (1.5), selects for sulfates over carboxyls
  - thus a correction for alginate
  - standard curve made with chondroitin-6 sulfate
- Typically normalize to cell amount (cf DNA content in qPCR)… maybe next year

ELISA protocol

- Direct ELISA uses labeled primary antibody
- Indirect ELISA – why use a secondary antibody?
  - flexibility/efficiency (t, $) → use w/many 1° Abs
  - signal amplification (Fc epitopes)
  - but maybe more cross-reactive
- Development process – what/why/how

  2° Ab has AP conjugate

  provide substrate: pNPP → colorimetric rxn.

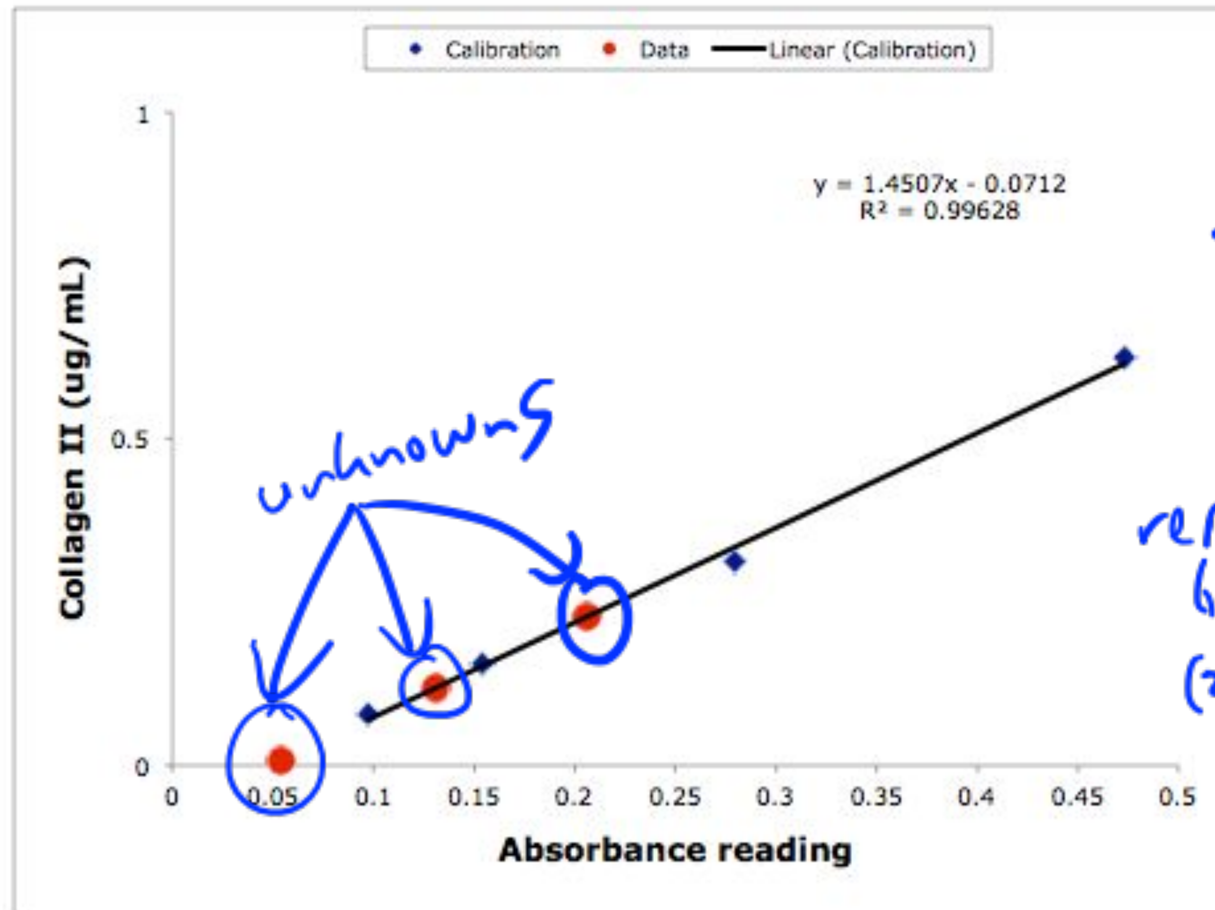
  insolu. A_{420}

  * development time is key ↗ detect low [protein] → long enough; ↘ avoid over-saturation (of all) → not too long

ELISA Outcomes

Outcome	Possible Explanations
High reading in "blank" samples	• cross-contamination from samples • skipped block step ★ incomplete washing
No signal at all (including standards)	★ flipped 1° Ab s • too high [Tween] • added wrong 2° Ab, or old
Saturated signal for some samples ↳ expt i	too concentrated → run dilution series usually

ELISA (and PG) analysis



exclude non-linear standards!

n~4

report:
(1) CN II : I ratios
(2) absolute [CN]
* if (2) is very low, (1) is meaningless!

qPCR analysis

$$\text{ratio} = \frac{(E_{target})^{\Delta CP_{target}(control - sample)}}{(E_{ref})^{\Delta CP_{ref}(control - sample)}}$$

efficiency of each primer set

CN I or II

change in crossing point ($Cp = C_T$)

18S rDNA

control = Sample A (−FN)

sample = Sample B (+FN)

we can know expression of CN II (A) vs. CN II (B) BUT NOT truly CN II (A) vs. CN I (A)

if $E_{tar} = 2.0$, $\Delta Cp_{tar} = 2$

$E_{ref} = 2.0$, $\Delta Cp_{ref} = 0$

$\frac{2^2}{2^0} = 4$-fold change

Equation 1 from M.W. Pfaffl, *Nucleic Acids Res* **29**:2002 (2001)

Today in Lab (M3D6)

- Finish ELISA – includes 90 min incubation

  figure out how much DEV SOLN your super-group needs

- Meanwhile…
  - DMMB assay staggered (15-20 min of work)
  - qPCR analysis
  - finish viability analysis if you haven't already
  - cross-group research discussion
  - (optional: start clean-up)