- Announcements
- Quiz
- Pre-lab Lecture
  - More about ELISA

Announcements

- Evaluations
  - WAC partly in class, end of Lec 7
  - Overall subject evaluation available online
- Mod 3 report
  - No separate methods section needed
  - State anything unique along the way in results section (alginate type, RNA recovered, etc.)
- Cross-group discussion today *or* next time

ELISA protocol

- Direct ELISA uses labeled primary antibody
- Indirect ELISA – why use a secondary antibody?
  - signal amplification
  - flexibility (use 20 primary different 1° efficient)

  but cross-reactivity more likely
- Development process – what/why/how

  2° Ab has enzyme = AP } colorimetric rxn.
  provide substrate = pNPP }

  amplification → development time is key

ELISA Outcomes

Outcome	Possible Explanations
High reading in "blank" samples	cross-contamination * incomplete washes forgot to block
No signal at all (including standards)	poor systemic binding ; inhibitor old reagents ; wrong 1° or 2° Ab too high [Tween]
Saturated signal for some samples	• too concentrated ∴ repeat w dilutions