COMPARISON OF VITAMIN B6 IMMUNOASSAYS

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MLSP 6905 Capstone Project Presentation

Project Research Question

- Relevance of vitamin B6/pyridoxal 5'-phosphate (PLP)
- Goal: determine which of 4 commercially available PLP quantitation kits performs best

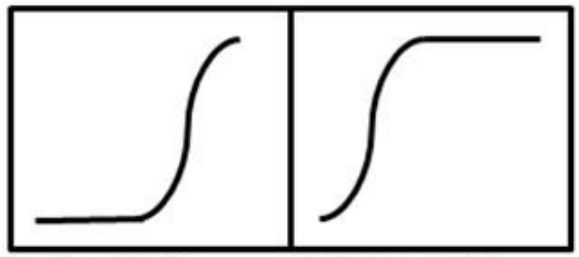
Literature Review / Background

- LC—MS is the most common method, followed by ELISA
- Alternatives exist, but may not be commercially available
- Some research is designed around the need to quantify PLP
- Focused on competitive ELISAs

Materials & Methods

- Tsai lab provided human EDTA plasma samples
- Preliminary testing was done in November
- Follow-up testing in December
- Kits were run according to manufacturer instructions
- Competitive ELISA principle

Results 1 – Preliminary comparison



Asymmetrical 5PL Curves

5PL Equation:

$$y = d + \frac{a - d}{\left[1 + \left(\frac{x}{c}\right)^{b}\right]^{g}}$$

a = theoretical response at zero concentration
b = slope factor
c = mid-range concentration (inflection point)
d = theoretical response at infinite concentration
g = asymmetry factor

Table 1: Number of samples within each assay's standard curve

B6 Assay Producer	Samples within 5PL standard curve
Biomatik	77 (96.25%)
Lifespan Biosciences	72 (90%)
Novus Biologicals	69 (86.25%)
MyBioSource	10 (12.5%)

Results 2

Table 2: Average coefficient of variation of duplicates by assay manufacturer

B6 Assay Producer	Average CV of duplicates
Biomatik	63.0%
Lifespan Biosciences	18.3%
Novus Biologicals	23.8%

Table 3: Average CV of dilutions by assay manufacturer

B6 Assay Producer	Average CV of dilutions
Biomatik	33.1%
Lifespan Biosciences	41.5%
Novus Biologicals	27.4%

Results 4

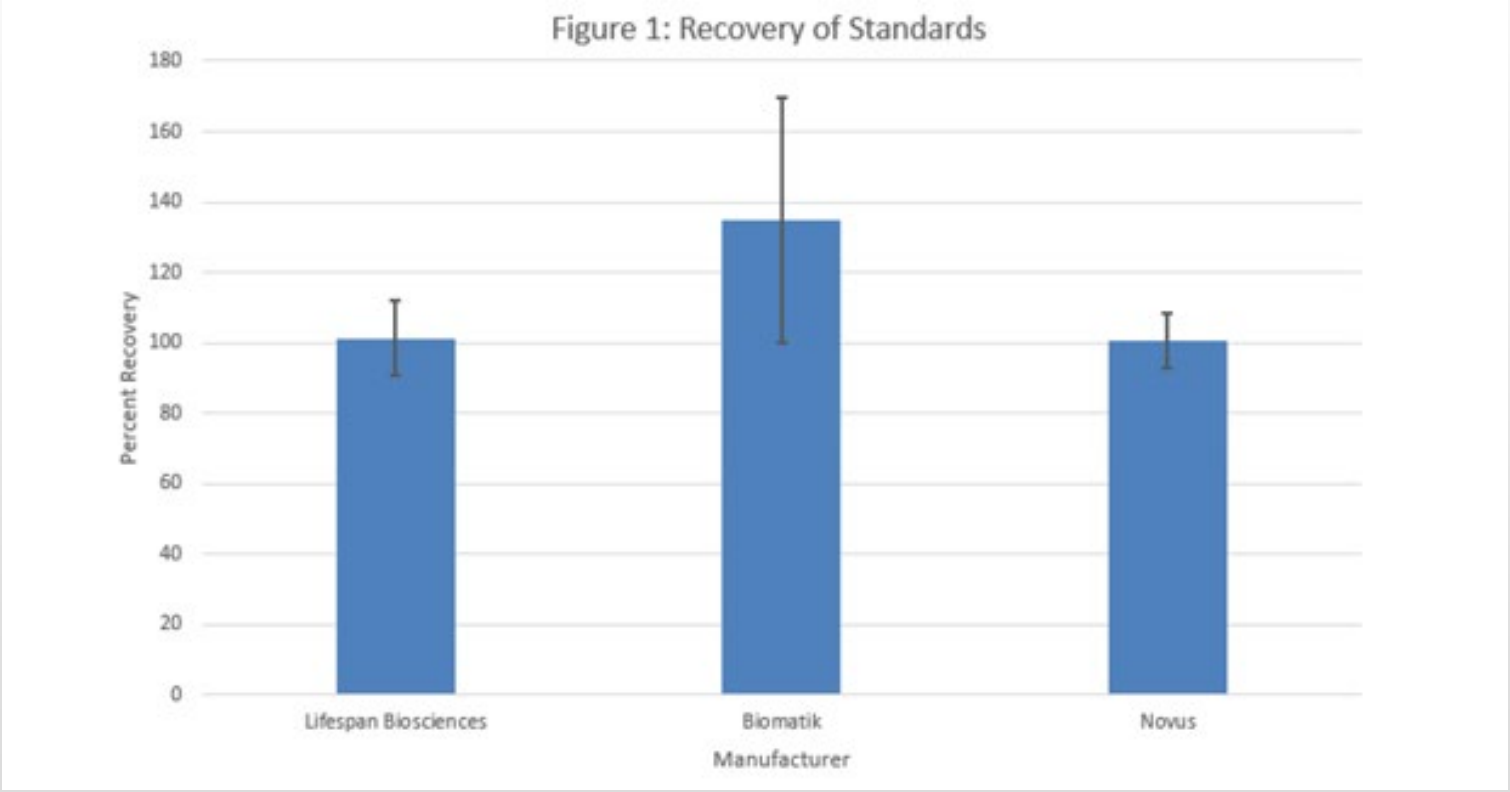


Figure 1: Recovery of Standards
180
160
140
120
100
80
60
40
20
0
Percent Recovery
Lifespan Biosciences
Biomatik
Novus
Manufacturer

Discussion / Conclusion

- Preliminary data → focus on Biomatik and Lifespan
  - Why did MBS perform so poorly?
- Standard concentration differences
- Rationale for final recommendation

Study Limitations / Next Steps

- Pilot study = low sample size
- Sample stability, freeze-thaw cycle
- Other methods (enzymatic)

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