Blood Phenylalanine Measurement in Phenylketonuria (PKU): A Comparison Between Plasma (PAA) vs. Dried Blood Spot (DBS)

Serei Vatana Nath[1], Caspar Lu[2], Rani H. Singh[1]

[1] Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA

[2] Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

https://mnt4p.org/

Background

- Phenylalanine (Phe) monitoring is essential for managing phenylketonuria (PKU)
- Phe levels can be measured using plasma or dried blood spot (DBS) samples
- Prior studies report inconsistent differences between specimen types
- Variability depends on methodology and laboratory techniques

Objectives

- Compare plasma and DBS phenylalanine levels using same methodology LC-MS/MS
- Evaluate agreement and systematic bias between methods
- Assess implications for clinical interpretation

Methods

Study Design

- Systematic literature review for meta-analysis of different lab methods – Ion exchange chromatography (IEC), tandem mass spectrometry (MS/MS), liquid chromatography tandem mass spectrometry (LC-MS/MS), high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), amino acid analyzer (AAA) – and different specimen types – plasma, dried blood spot from capillary (DBSC) and from vein (DBSV).
- Comparative analysis of plasma vs dried blood spot (DBS) samples

Measurement:

- Phenylalanine quantified using LC-MS/MS

Sample Collection

- Paired plasma and DBS samples collected at Emory MNT4P Metabolic Camp 2024
- Samples analyzed under the same conditions

Statistical Analysis

- Bland–Altman analysis → bias & agreement
- Concordance correlation coefficient (CCC)
- Regression models for bias correction

Total campers with PKU who attended Emory Metabolic Camp 2024 (N = 31)

↓

Campers with pre- and post-camp plasma DBS available (N = 18)

↓

Campers with complete/valid pre- and post-camp plasma and DBS (N = 17)

↓

Pre- and Post-camp data were combined/pooled

↓

Total sample size for plasma (N = 34) and DBS (N = 34)

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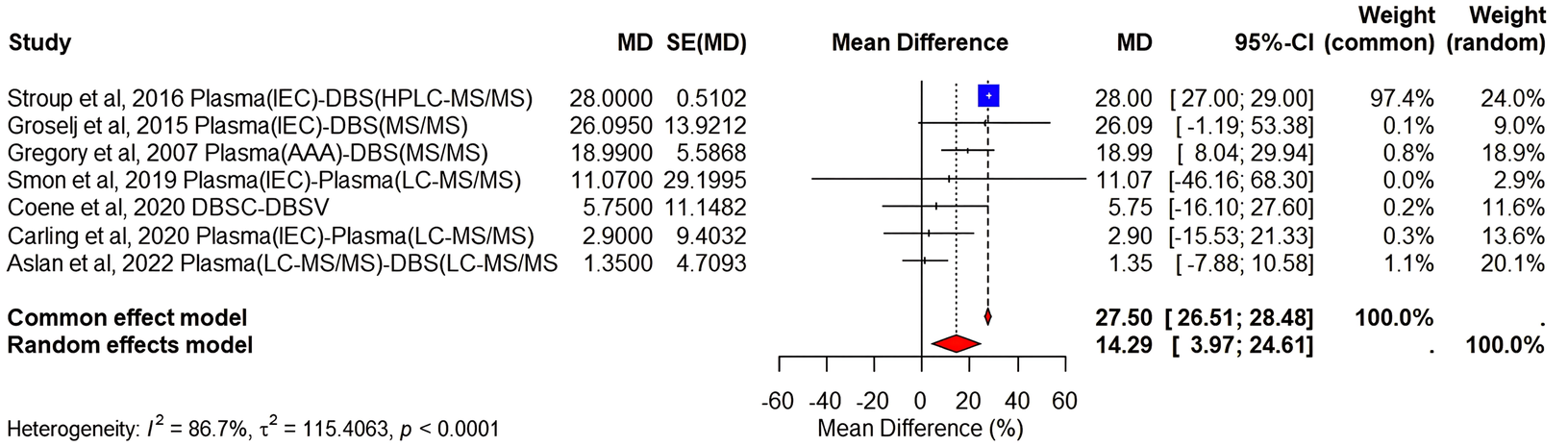
Results

Figure 1. Meta-analysis of Plasma vs DBS Phenylalanine Differences



Heterogeneity: I^2 = 86.7%, τ^2 = 115.4063, p < 0.0001

Meta-analysis Shows High Variability: Pooled mean difference: **27.5%** (fixed-effects**) 14.3%** (random-effects); High heterogeneity: **I^2 = 86.7%, p < 0.000;** Differences likely driven by methodological variation. Differences between plasma and DBS are consistent but highly variable across studies.

Table 1. Sample Collected during MNT4P Metabolic Camp 2024

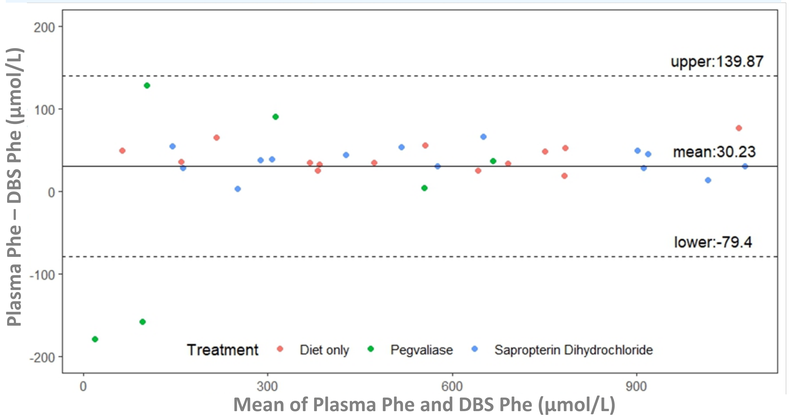
Specimen Types	N	Mean	SD	*p*
Dried Blood Spots (DBS)	34	408	252	***
Plasma Amino Acid (PAA)	34	604	380	***

[a]Statistical significance was calculated with Wilcoxon signed-rank test. *** p< .001.

[b]Phe concentration was noted as (µM)

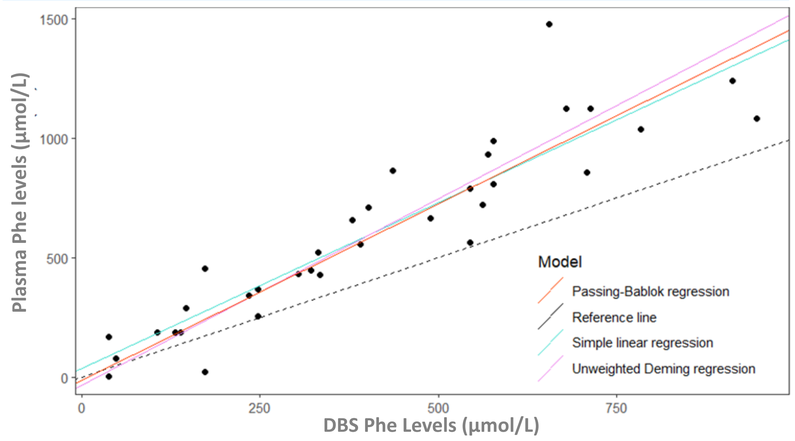
Plasma phenylalanine levels were significantly higher than DBS despite measurement using the same LC-MS/MS method (p < 0.001).

Figure 2. Bland-Altman Analysis of PAA vs. DBS



Bland–Altman analysis shows a ~30% positive bias, with plasma values generally higher than DBS and wide limits of agreement (−79.5% to 139.9%) indicating substantial variability. Variability appears greater at lower end .

Figure 3. Systematic and Proportional Bias Corrections



Model	Estimate	Lower CI.	Upper CI.
Raw data	0.7121	0.5753	0.8101
Simple linear	0.917	0.8428	0.957
Deming unweighted	0.9196	0.8458	0.9589
Passing-bablok	0.9194	0.8453	0.9588

[a]Agreement rates was calculated with Concordance Correlation Coefficient.

[b]Coefficients were transformed with Fisher's Z transformation.

[c]CI. indicates 95% confidence interval for Z distribution.

Plasma phenylalanine correlates with DBS phenylalanine but is systematically higher, requiring correction for comparison. The concordance correlation coefficient (CCC) was 0.71 (95% CI: 0.58–0.81), indicating moderate agreement, and improved to strong agreement after bias correction using regression models.

Conclusions

- Plasma phenylalanine levels are higher than DBS across the measurement range.
- A consistent systematic bias (~30%) affects comparability
- Adjustment methods improve agreement
- Clinical interpretation of DBS values should account for this bias

Standardization or correction factors are needed for accurate PKU monitoring.