Important lessons on instability of amino acids in dried blood spots

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BACKGROUND

Stored heel prick Dried Blood Spots (DBS) are valuable samples for retrospective investigation on inborn errors of metabolism (IEM), including post-mortem investigations, biomarker evaluation and validation studies.

However, many metabolites are subjected to degradation during long-term storage. Moreover, storage conditions vary worldwide and within countries. Data on amino acid stability is therefore essential for proper interpretation of DBS investigations.

AIM

This study aimed to investigate the 5-year stability of amino acids in stored heel prick DBS.

METHODS

Data was used from the retrospective BURDEN study (1). Amino acids profiles were analyzed for 2170 anonymous DBS collected and stored from 2013-2017. DBS were stored at +4 degrees during first year and at room temperature thereafter at the RIVM, and at -20 °C in our laboratory (see Fig 1).

Amino acids were analyzed in random order in 2019-2020 using a validated LC-MS/MS method. Observations above 1.5 IQR were considered outliers and were excluded from analysis.

Amino acid stability was assessed by principle component analysis (PCA) to assess year cohorts differences, by annual amino acid decays over the years and by Jonckheere’s-Terpstra tests.

Amino acids were considered stable when the 5 year change was < max percental change, based on the (amino acid specific) sum of the biological and analytical variation. Amino acids were instable when the 5 year change was > max change, and partly stable when stable for 1-5 years.

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Figure 1. Storage conditions DBS
RESULTS (1/2)

The PCA plot shows gradual differences between the year cohorts.

Figure 2: PCA score scatter plot of amino acid concentrations in 2170 DBS. Data outside the ellipse represent outlier data. Data was UV-scaled, meaning that each scaled variable has equal variance (Umetrics).

MAIN RESULTS

Over the course of 5 years, from least to most stable, concentrations of:

- **Group 1 (unstable):** glutamine, tryptophan, taurine, lysine, asparagine, glycine, ornithine, serine, threonine, phenylalanine, tyrosine, leucine, valine, isoleucine **decreased significantly.**

- **Group 2 (partly stable):** alanine, glutamate and proline remained **stable up to 2 years.**

- **Group 3 (stable):** citrulline, aspartate and hydroxyproline remained **stable for 5 years.**

**Example unstable amino acid: Glycine**

Figure 3. Example of an unstable amino acid: Annual means of glycine. Legend: A) annual means representing **annual decay** in metabolite concentration B) absolute increase in metabolite concentration.
RESULTS (2/2)

Example partly stable amino acid: Alanine

Figure 4. Example of a partly stable amino acid: Annual means of alanine. Legend: A) annual means representing annual decay in metabolite concentration B) absolute increase in metabolite concentration

Example stable amino acid: Citrulline

Figure 5. Example of a stable amino acid: Annual means of citrulline: Legend: A) annual means representing annual decay in metabolite concentration B) absolute increase in metabolite concentration
DISCUSSION & CONCLUSION

Instability of amino acids can cause **incorrect interpretation** of test results from stored BDS.

This impacts retrospective biomarker studies and IEM diagnostics.

In order to avoid these problems, we propose proper DBS **storage at -80 degrees** in humidity-controlled conditions.

If this is not possible, we recommend use of control DBS

**References**

Related articles:

Van Rijt WJ et al. Inborn errors of metabolism that cause sudden infant death: a systematic review with implications for population neonatal screening programmes.


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