Development and Validation of a Multiplexed Dried Blood Spot Flow-Injection Analysis Method for Quantitation of Amino Acids, Acylcarnitines, Organic Acids, Purines and Lysophosphotidylcholines used in First-tier Newborn Screening in Ontario

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Objectives

Quantitative analysis of amino acids and acylcarnitines (AAAC) in dried blood spots (DBS) is an essential component of all modern newborn screening (NBS) systems. The use of a butylation step in the sample processing of AAAC assays was once a common practice but with improvements to mass spectrometer sensitivity and sample processing, many programs are moving to non-butylated assays. Newborn Screening Ontario (NSO) still uses butylation in the derivatized approach to AAAC screening. NSO has been mandated to add X-Linked Adrenoleukodystrophy (X-ALD) screening in 2025. Implementation of X-ALD screening typically involves multiplexing into first-tier AAAC methods, however the acidic environment required for butylation causes C26:0LPC hydrolysis. NSO has developed and piloted a first-tier small molecules (SM1ST) assay without butylation to accommodate the addition of X-ALD screening.

Methods

Sample preparation involves excising single 3.2mm DBS punches into 96-well plates. A double extraction is performed, first using a methanolic solution containing labeled internal standards and second acetonitrile-water solution. The eluent is transferred to a new plate and 10 µL analyzed. NBS samples, linearity materials from the Center for Disease Control, QC materials from Astoria Pacific, Internal Standards from Cambridge Isotope Laboratories and Renata Screening Systems from Waters were used to assess linearity, precision, comparability, LOB, LOD and LOQ.

Results

A pilot phase where >25,000 NBS, >900 QC and >100 linearity samples were analyzed on both AAAC and SM1ST assays. Analyte specific response factors were established, matching SM1ST quantitation to AAAC, allowing use of established screening cut-offs and reference intervals for most primary analytes. Problematic or new analytes, including Suac, ASA and C26:0LPC, required alignment to CDC linearity materials.

Conclusions

NSO successfully developed and validated a non-butylated approach to first-tier small molecules screening that will allow the multiplexing of all current AAAC analytes and C26:0LPC. The assay will go-live on September 29, 2025.