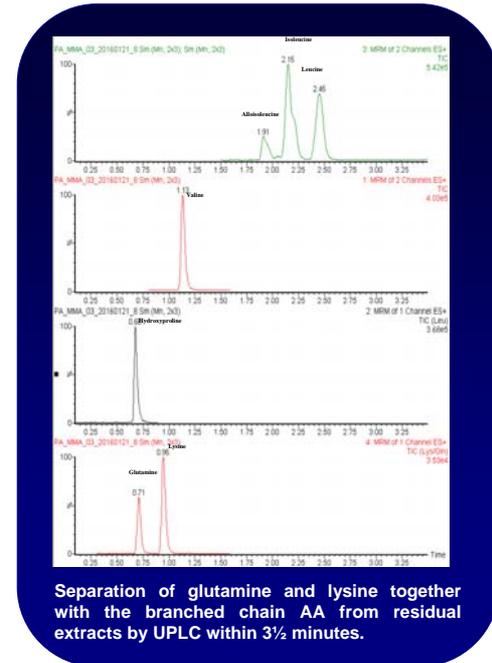


Introduction

The inclusion of urea cycle disorders (UCD) into newborn screening (NBS) is highly desirable but hampered by the lack of specific markers. Exceptions are citrulline and argininosuccinate (ASA) for detection of citrullinemia and argininosuccinic aciduria, respectively. So far, the common feature of all UCDs, hyperammonemia, is not directly detectable in dried blood spots (DBS), and the quantification of secondary elevations of glutamine was thought to be not feasible based on the assumption of instable glutamine in DBS.

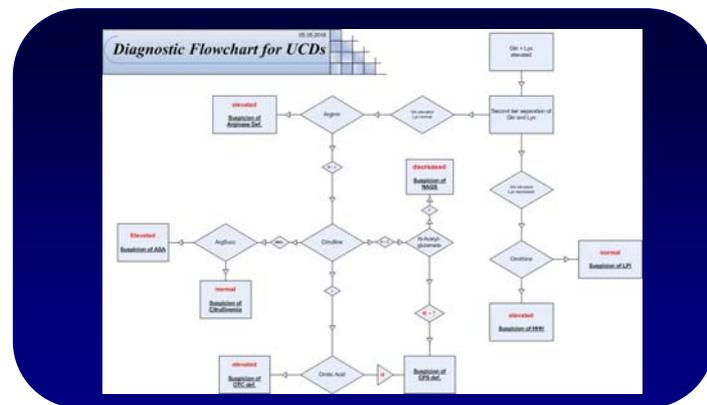
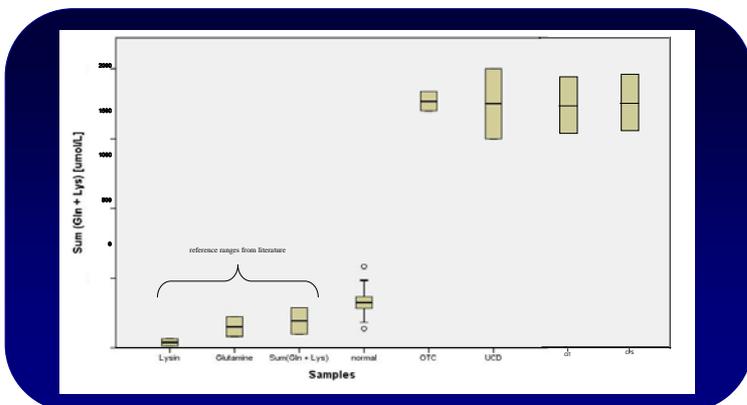
Results

388 anonymised samples from healthy newborns were tested. The median and 99th centile for the sum of glutamine and lysine were 212 and 958 μM , respectively. 103 samples were additionally separated by UPLC. Median and 99th centile were: 65 and 534 μM (lysine), and 507 and 899 μM (glutamine). In addition 5 samples from 4 patients with UCDs were analysed: 3 samples from 2 patients with OTC deficiency, 2 from a patient with citrullinemia, and 1 from a patient with CPS-I deficiency. The sum of glutamine and lysine ranged between 1500-2500 μM , and glutamine (after UPLC) ranged between 1000-2500 μM . Orotic acid was only elevated in the samples from OTC patients.



Methods

The NeoMass AAAC test kit from **Labsystems Diagnostics** was used together with a Waters Xevo TQD tandem-MS and a Waters Aquity H-Class UPLC. Glutamine and lysine were detected simultaneously from DBS in multiple reaction monitoring (MRM) with a second-tier UPLC-method for the separation and specific quantification of glutamine (Fingerhut et al. *Int. J. Neonatal Screen.* 2016, 2(2), 2). We combined this newly developed method with the measurement of all relevant amino acids (arginine, ASA, citrulline, ornithine, and proline), and orotic acid.



Conclusion

We describe a reliable and sensitive method for the detection of **all** UCDs by TMS-NBS. The next step will be a prospective study with DBS samples from patients with hyperammonemia, allowing further testing and evaluation of the method in practice.