Combined tandem mass spectrometry (TMS) screening method for Biotinidase Deficiency, Tyrosinemia Type I (HT1) and Sickle Cell Disease (SCD)

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Aim

- To save valuable patients material as well as personal and technical resources by combination of enzymatic testing for biotinidase deficiency and tyrosinemia type I with sickle cell disease screening using tandem mass spectrometry
- To demonstrate the suitability of the new method by comparing with methods used in a routine lab

Materials and Methods

Samples

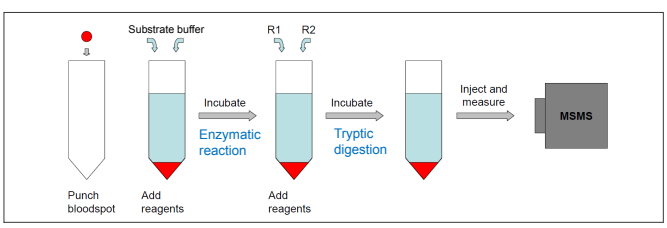
37.000 residual newborn screening samples

- Restricted to samples taken at a corrected gestational age > 32 weeks and > 36 h of life
- Stored at r.t.; Sample preparation between day 15 and 30 (min 11, max 64 days)

Sample preparation

Adapted SpotOn Clinical Diagnostics dried blood spot (DBS) TMS method for SCD screening[1]:

1. Elution with substrate buffer containing biocytin and 5-aminolevulinic acid
2. tryptic digestion of hemoglobin



Mass spectrometry

4000 QTRAP (Sciex) with HTS PAL autosampler (CTC Analytics) and Prominence HPLC (Shimadzu)

Flow injection of 2µl sample and ESI+ detection in MRM mode of

- Characteristic peptides of HbA, HbS and HbC
- Biocytin and Biotin
- 5-Aminolevulinic acid (5-Ala) and Porphobilinogen (PBG)

Routine methods

Biotinidiase

Classical method by Heard et al.[2] with biotinyl-p-aminobenzoate as substrate and photometric detection of p-aminobenzoate (PABA)

Succinylaceton (SUAc)

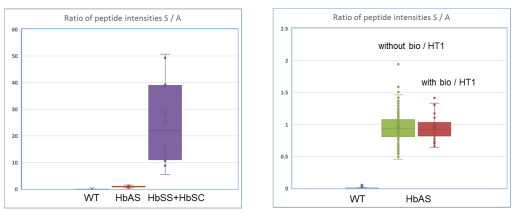
As hydrazone derivative by TMS using the kit Amino acids and Acylcarnitines non-derivatised (Chromsystems) with supplemental kit for SuAc

[1]Daniel Y et al (2005) Rapid and specific detection of clinically significant haemoglobinopathies using electrospray mass spectrometry-mass spectrometry. *BJH, 130, 635-643*

[2]Heard et al (1984) A Screening Method for Biotinidase Deficiency in Newborns. *Clin. Chem. 30/1, 125-127*

Results

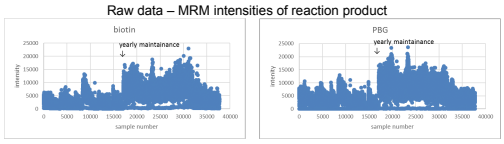
SCD – use of intensity ratio S/A for HbS and HbA peptides



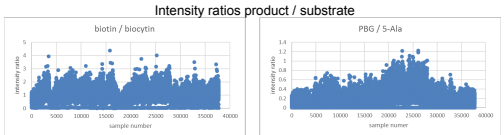
Number of samples: WT 8000; HbAS: 229 / 39 (without / with Biotinidase / HT1 testing); HbSS + HbSC: 12 (3/9)

Enzymatic testing – Development of data evaluation strategy

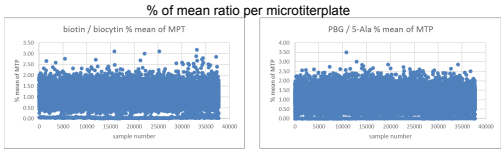
Raw data – MRM intensities of reaction product



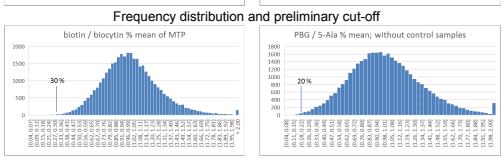
Intensity ratios product / substrate



% of mean ratio per microtiterplate

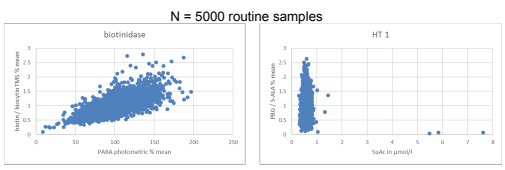


Frequency distribution and preliminary cut-off

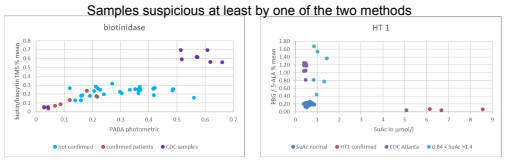


Comparison with routine methods

N = 5000 routine samples



Samples suspicious at least by one of the two methods



Summary

- Sickle cell disease screening is not influenced by enzymatic reactions preceeding the tryptic digest
- All true positives for biotinidase deficiency and HT1 were correctly assessed by the new TMS method
- Although results of both methods correlate well, selectivity of the new enzymatic TMS method seems to be slightly lower, probably due to restrospective examination of samples stored at r.t.
- Within a cohort of 37.000 newborn samples were found:
  - 8 newborns affected by sickle cell disease
  - 2 complete and 3 partial biotinidase deficiencies
  - 1 Tyrosinemia Typ1

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