

Newborn Screening for hereditary tyrosinemia type I: succinylacetone isolation without hydrazine

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ABSTRACT AND INTRODUCTION

Newborn screening for hereditary tyrosinemia type I (HT 1) is mandatory to identify infants at risk before life-threatening symptoms occur [1–3]. The analysis of tyrosine alone is limited, and might lead to false-negative results. Consequently, the analysis of succinylacetone (SUAC) is needed [1-4]. Current protocols are time-consuming, and above all, include hazardous reagents such hydrazine. We evaluated a novel, commercial kit to analyze amino acids, acylcarnitines and SUAC with a significantly less harmful hydrazine derivative in a newborn screening laboratory. Dried blood spot (DBS) specimens from 4,683 newborns and samples from known patients with inborn errors of metabolism (IEM) were analyzed by a novel protocol and compared to an in-house screening assay. All samples were derivatized with butanol-HCl after extraction from 1/8-inch DBS punches. For the novel protocol, the residual blood spots were extracted separately for SUAC, converted into hydrazone, combined with amino acids and acylcarnitines, and subsequently analyzed by mass spectrometry using internal isotope-labeled standards. All newborns were successfully tested, and 74 patients with IEMs including three HT 1 (SUAC 1.50, 4.80 and 6.49; tyrosine levels 93.10, 172.40, and 317.73, respectively) were detected accurately. The mean SUAC level in non-affected newborns was 0.68 µmol/l (cut-off 1.29 µmol/l). The novel assay was demonstrated to be accurate in the detection of newborns with IEM, robust, and above all, without the risk of the exposure to highly toxic reagents and requirement of additional equipment for toxic fume evacuation.

MATERIAL AND METHODS

For the newborn screening 4,683 blood samples were collected from neonates consecutively in an one-month study period during the national routine Austrian Newborn Screening Program. Samples that were screened successfully for the regular screening panel for endocrine and metabolic disorders, including cystic fibrosis [5], were analyzed in parallel with a novel, validated commercial kit (*MassChrom*® Amino Acids and Acylcarnitines from Dried Blood; Chromsystems, Gräfelfing/Germany) at the Department of Paediatrics and Adolescent Medicine, Medical University of Vienna/Austria.

This kit was used for the semi-quantitative determination of amino acids, acylcarnitines and SUAC from one single DBS [6]. The additional mass spectrometric analysis included 40 analytes (13 amino acids, carnitine, 25 acylcarnitines, SUAC). The measurements were performed on a TQD mass spectrometer (Waters, Milford/MA USA) with electrospray-ionisation in positive ion mode. The analysis was valid whenever the controls (quality control material provided by Chromsystems) were in range. In cases where quality controls did not match, the complete analysis for this batch was repeated. DBSs from potentially affected newborns were re-tested in duplicate, and in the case of positivity, diagnostically confirmed in accordance with the institutional guidelines.

RESULTS

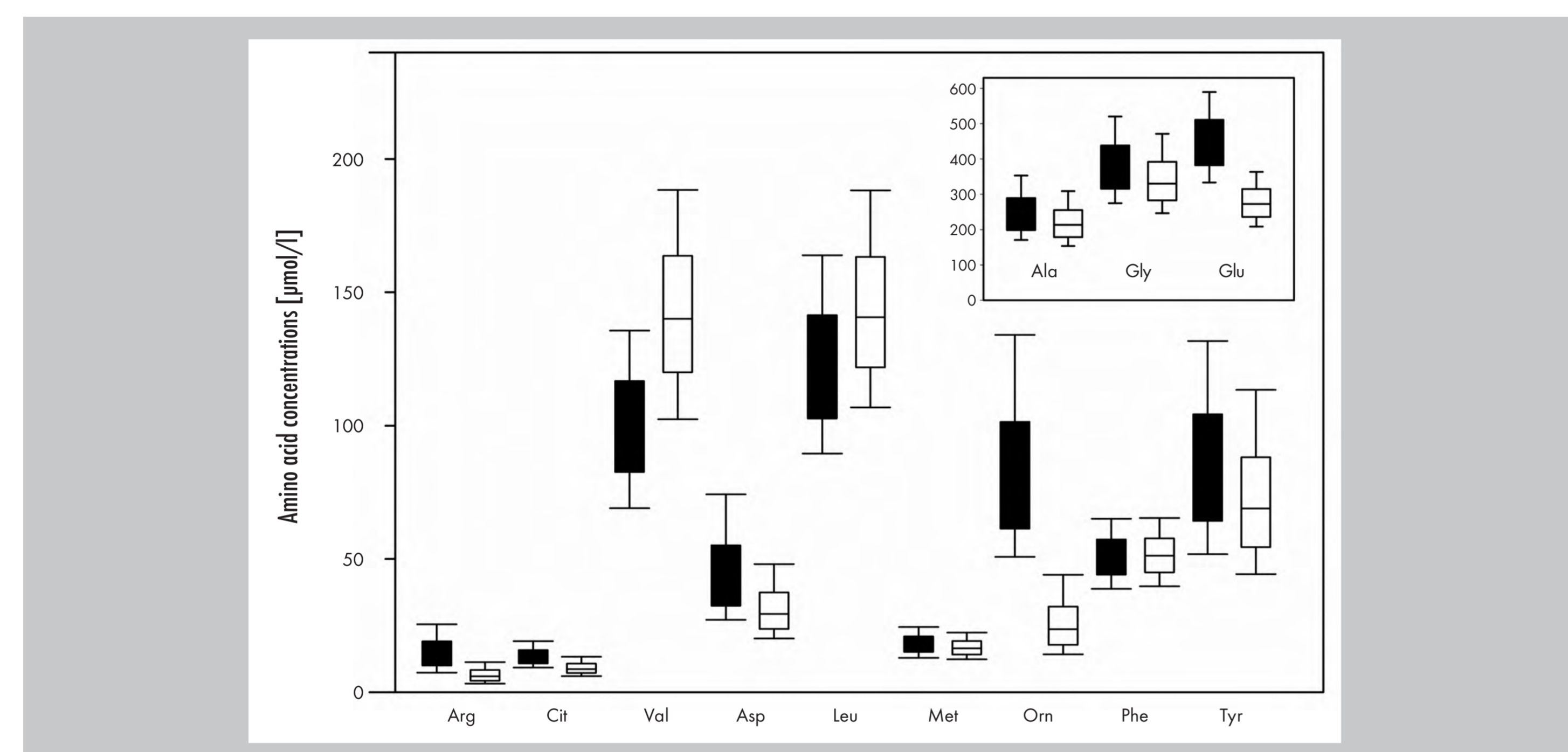


Figure 1: Method comparison of two mass spectrometry protocols for amino acid analysis. Black boxes: novel kit including SUAC from Chromsystems, white boxes: reference method.

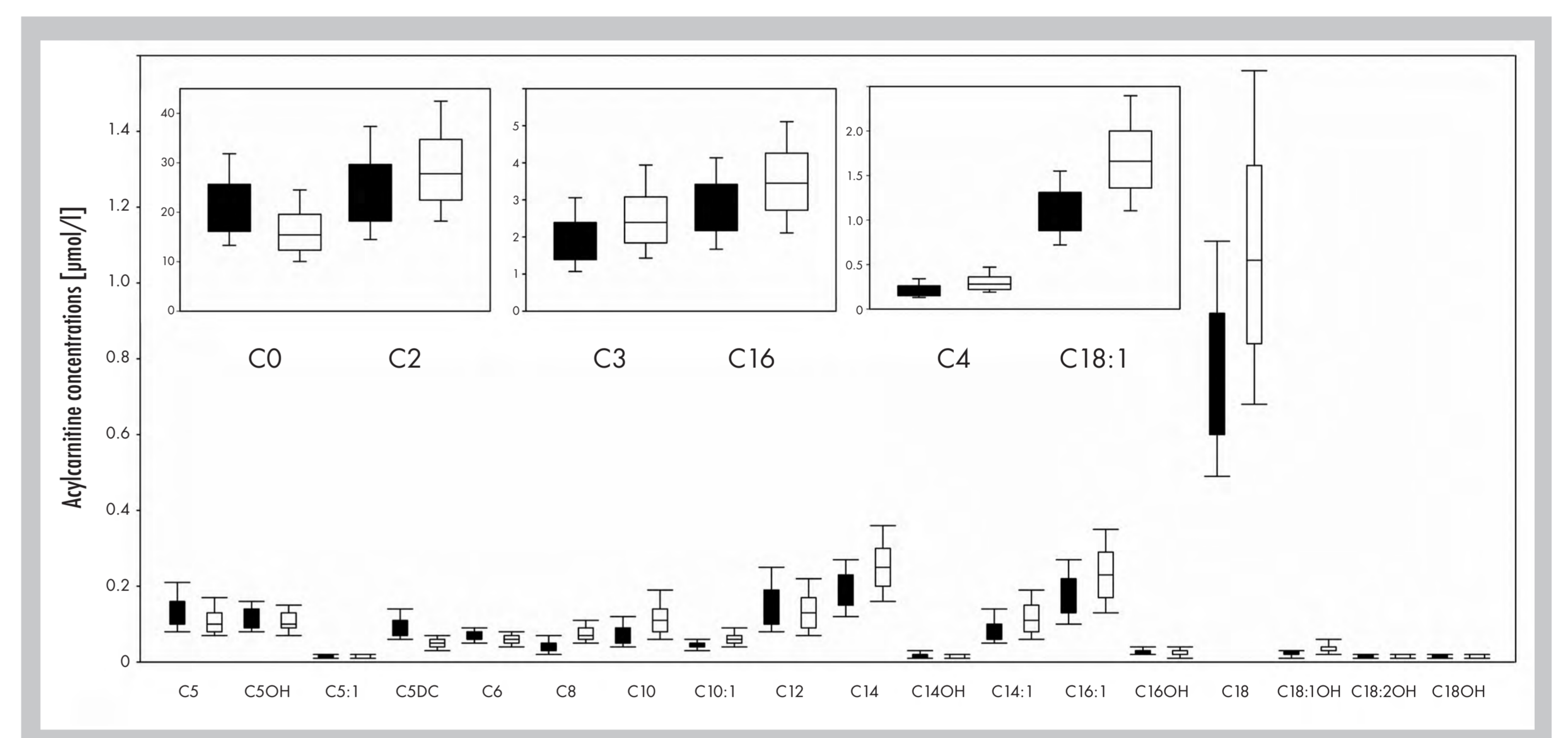


Figure 2: Method comparison of two mass spectrometry protocols for acylcarnitine analysis. Black boxes: novel kit including SUAC from Chromsystems, white boxes: reference method.

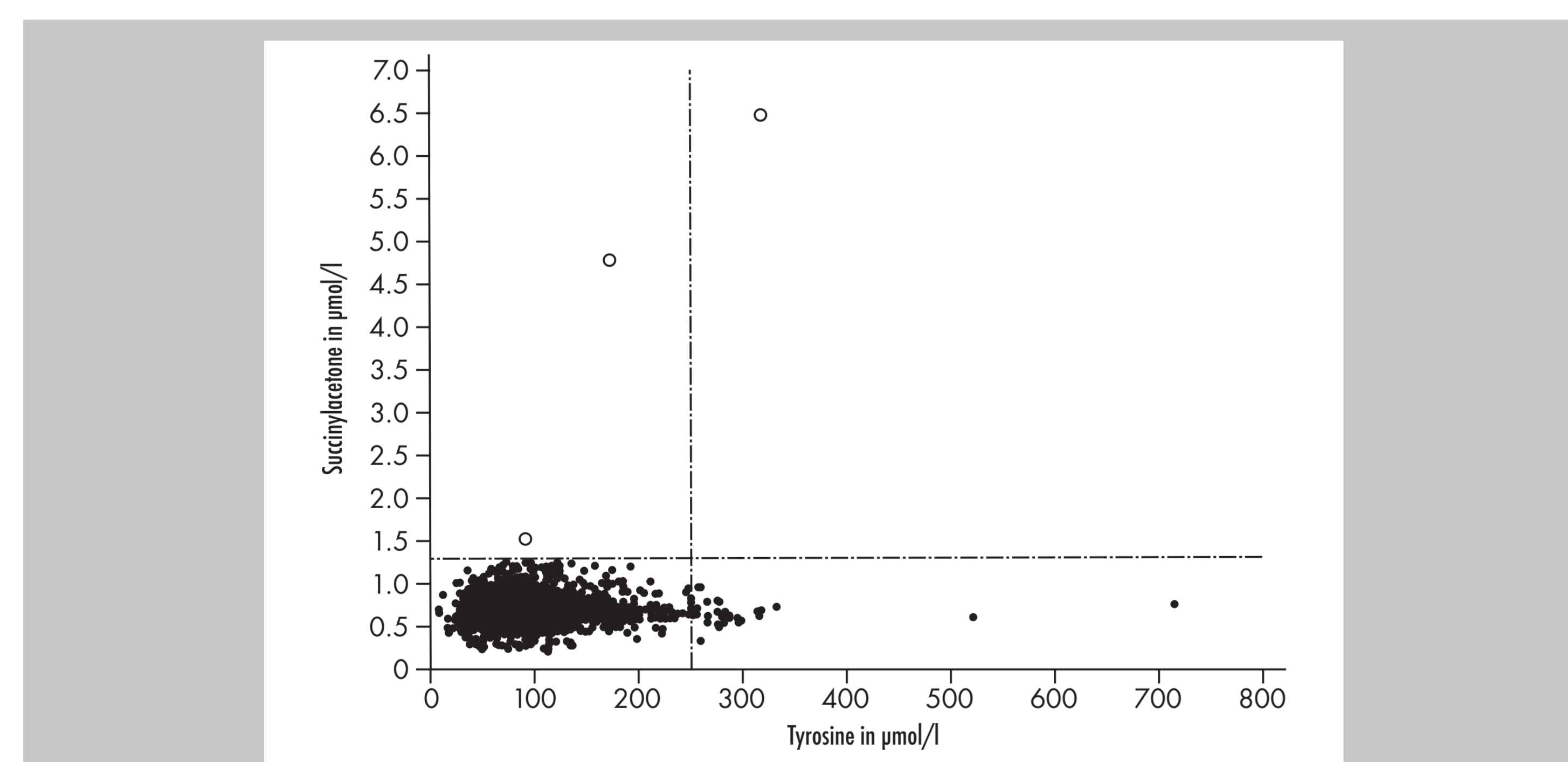


Figure 3: Scatter plot of succinylacetone vs tyrosine levels in non-affected newborns and patients with tyrosinemia type I. White dots represent three patients with HT1 (cut-off for SUAC: 1.29 µmol/l, for Tyr: 255 µmol/l). Black dots: non-affected newborns.

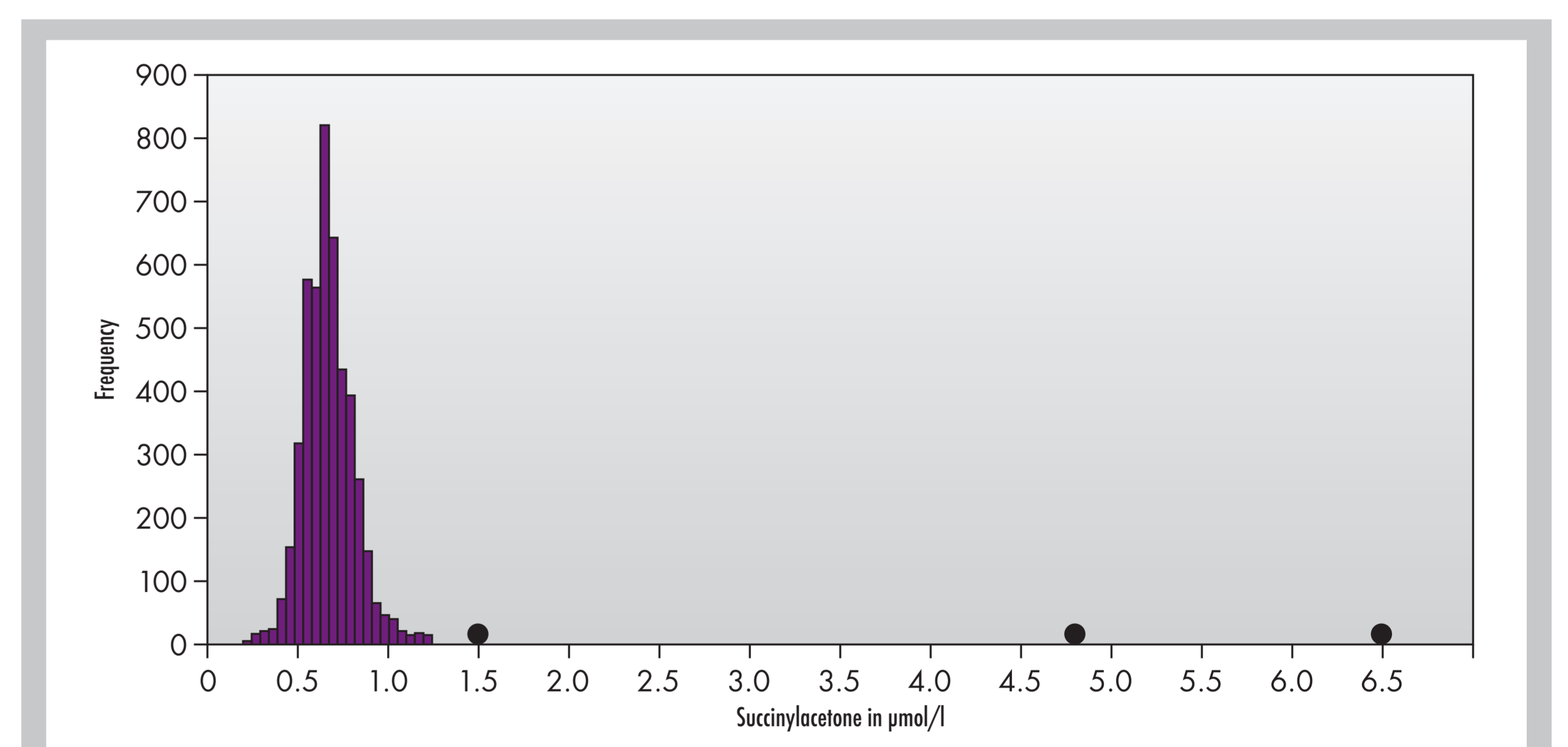


Figure 4: Distribution of SUAC levels in newborns (n= 4,683). Black dots represent three HT 1 patients with elevated SUAC levels.

CONCLUSION

- Tyrosine as the only marker is insufficient to verify HT 1. Inclusion of SUAC in newborn screening programs should increase the safe identification of HT 1 in neonates and minimise false positive or false negative results.
- Beside other IEMs all three patient samples with the known target disease HT 1 were identified according to the significant elevated HT 1 specific marker SUAC with the new Chromsystems' *MassChrom*® Amino Acids and Acylcarnitines from Dried Blood kit.
- The novel assay was demonstrated to be sensitive, rapid, and robust, and above all, without the risk of the exposure to highly toxic reagents.

References:

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