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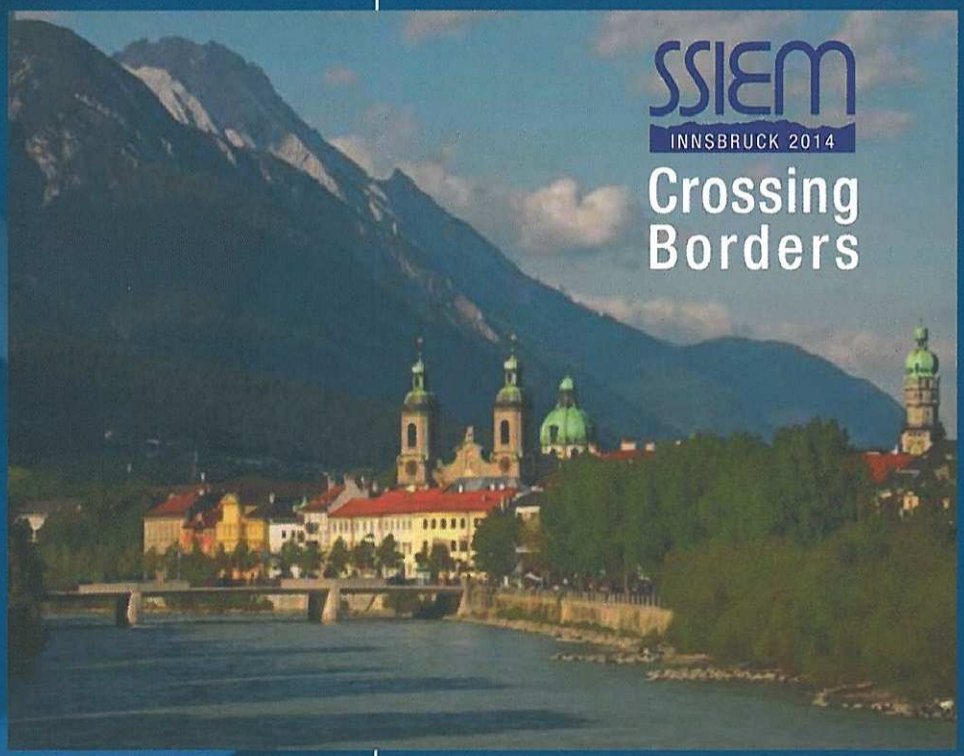

ABSTRACTS

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Interestingly, we have extended the ability of the kit with the addition of 8 amino acids which are: sulfocysteine, pipecolic acid, aspartylglucosamine, homocarnosine, glutathione, saccharopine, cysteine-homocysteine mixed disulfides and iminodipeptides. Firstly, we describe the determination of iminodipeptides and arginosuccinic acid which can be analysed without previous hydrolysis. Secondly, we show the detection of pipecolic acid which is particularly important in diagnosis of peroxisomal disorders.

P-010

A capillary electrophoresis procedure for the screening of oligosaccharidoses and related diseases

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Background: The most widely used method for the biochemical screening of oligosaccharidoses is the analysis of the urinary oligosaccharide pattern by thin-layer chromatography. Our aim was to standardize the analysis of urine oligosaccharides by capillary electrophoresis with laser-induced fluorescence (CE-LIF).
Methods: CE-LIF (Beckman P/ACE MDQ) was equipped with a 488 nm argon ion laser module. All analyses were conducted using the Carbohydrate Labelling and Analysis Kit (Beckman-Coulter), which derivatizes samples with 8-aminopyrene-1,3,6-trisulfonate. Urine samples from 40 control subjects (age range: 1 week to 16 years) and from 10 patients diagnosed with eight different oligosaccharidoses (6 of them included in the Educational Oligosaccharide Kit from ERNDIM EQA schemes) were analysed.
Results: Two oligosaccharide excretion patterns were established in our control population according to age. Abnormal peaks with electrophoretic mobilities above the tetrasaccharide position were observed for fucosidosis, α-mannosidosis, GM1 gangliosidosis, GM2 gangliosidosis variant 0, Pompe disease and glycogen storage disease type 3. Urine from patients with aspartylglucosaminuria and Schindler disease displayed normal results.
Conclusions: In this study, the first CE-LIF method to screen for oligosaccharidoses and related diseases has been standardized. The method is simple, fast, automatable and allows the analysis of large series of samples.

P-011

High resolution nuclear magnetic resonance spectroscopy (NMRS): metabolic profiling in urine in healthy children

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Background: NMRS profiling in body fluids has become a powerful tool to investigate genetic metabolic diseases. Metabolic profiling using NMRS, however, needs reference profiles from healthy individuals since deviations from the "normal" state can only be detected using multivariate statistical analysis on base of reference profiles.
Patients and methods: 120 healthy children (age 0-15years) were recruited from an outpatient medical center to investigate NMRS profiles in spontaneous urines. Somatic, nutritional and clinical data were documented. The samples were measured with an Advance IVDr system at 600 MHz.
Results: Different metabolic profiles were found showing an age dependency with higher excretion (based on creatinine) of metabolites in younger children. Selected metabolites were quantified and respective distributions were compiled to obtain reference ranges. Preliminary results in PKU patients demonstrate possibility to monitor compliance of treatment by metabolic profiling in urine.
Conclusion: NMRS profiling and quantification in urine using high resolution NMRS offer new aspects in metabolic research and monitoring of treated patients with genetic metabolic diseases. Methodological advantages of NMRS over HPLC/MS/MS or GC/MS are short analysis time, simple sample preparation. Additionally, NMRS in body fluids is fully quantitative at a large linear range of up to 6 orders of magnitude.
Conflict of Interest declared.

P-012

Validation of oxygen consumption measurements in muscle and fibroblasts from patients with mitochondrial diseases

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Background: Diagnosis of mitochondrial disorders is mainly based on the analysis of OXPHOS complexes in muscle biopsies. However, normal enzyme activities do not exclude such diagnosis. Analysis of the intact mitochondrial energy generating system by oxygen consumption is frequently used.
Purpose: Comparing the diagnostic value of oxygen consumption versus OXPHOS analysis in fibroblasts and muscle from patients with a genetically confirmed mitochondrial disease.
Methods: A standardized substrate uncoupler inhibitor titration (SUIT) protocol was used for measuring respiration of permeabilised fibroblasts and single muscle fibers. OXPHOS activities were determined spectrophotometrically according to standard protocols.
Results: Only the combination of both measurements enable us to identify all of the patients: In fibroblasts 4/15 patients would have been missed by OXPHOS measurements, but could be identified with oxygen consumption. In muscle 1/6 patients was missed by oxygen consumption, but was identified by OXPHOS measurements. Moreover, specific flux control ratios showed higher diagnostic than complex specific O2 fluxes.
Conclusion: The established SUIT protocol is an important tool in the diagnostic process. Therefore we recommend oxygen consumption measurements in addition to OXPHOS analysis, to increase the number of identified patients.

P-013

A practical enzymatic assay for determination of propionyl-CoA carboxylase activity using high-performance liquid chromatography

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Background: Propionic acidemia (PA) is a life threatening inborn error of organic acid metabolism caused by a defect of propionyl-CoA carboxylase (PCC). Prompt diagnosis and appropriate dietary restriction may prevent acute metabolic decompensation and brain damage. The measurement of PCC activity by a radioisotopic method is still the gold standard for the diagnostic confirmation of the disease. To realize a practical test, we developed a simple and rapid enzymatic assay for PCC.
Methods: Crude PCC enzyme was prepared from frozen-thawed human lymphocytes. Aliquots were incubated with propionyl-CoA, sodium biocarbonate and adenosine triphosphate. The production of methylmalonyl-CoA in samples was separated by high performance liquid chromatography and detected using an ultraviolet spectrophotometer.

Results: The assay was applied to four patients with PA and demonstrated pathologically low levels of residual activity in all subjects. There were no significant differences of enzyme activity between the obligated carriers, the parents of PA patients (221–365 pmol/min/mg protein, n=5), and normal individuals (170–575 pmol/min/mg protein, n=20).
Conclusion: These results indicate that our method is a practical and sensitive assay for PCC and that it can be a useful adjunct in confirm diagnosis of PA.

P-014

Rapid and accurate determination of phenylalanine with advanced aptamers in a hand-held device

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Background: Aptamers are oligonucleotide based receptors that were discovered 1989. Aptamers have a high target specificity, provide a direct readout after binding, are temperature independent and cheap to manufacture. We developed advanced aptamers with an up to 1000-fold binding affinity that enables detection of amino acid and other small molecules for the first time. This platform technology is amenable to point-of-care (PoC) devices.
Objective: Evaluate advanced aptamers for phenylalanine determination in a PoC device
Method: 20 ul serum is diluted 1:600, incubated for 20 min with advanced aptamers (0.05 uM) and read in a hand-held fluorescent reader against a standard curve.
Results: Range: 30–2000 uM. Limit of detection: 20 uM. Linearity R^2= 0.99 (30–720 uM range). Linear least-squares regression analysis demonstrates a high degree of correlation between spiked plasma and serum over the clinically relevant range (30–720 uM): y=0.8001x +185.4, R^2= 0.97. (Whole range (0–2000 uM): y=0.9494x +53.63, R2=0.89)). 25 patient samples were analyzed with HPLC and aptamers. Mean phe 69.8 uM (Range 42–124), constant bias: −14.67 (95 % CI; −40 to 1.23), proportional bias: 1.29 (1.003 to 1.67 95 % CI) (Passing & Bablok fit).
Conclusion: Advanced aptamers enable accurate measurement of phe.
Conflict of Interest declared.

P-015

Analysis of bile acid profiles by liquid chromatography-mass spectrometry

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Background: Bile acids are the major products of cholesterol catabolism. They serve many important physiological functions such as cholesterol homeostasis and lipid digestion and absorption. In this work we focus on bile acid profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in patients with necrotizing enterocolitis (NEC) and possible peroxisomal disorders.
Methods: Bile acid extraction from serum was performed by protein precipitation with methanol, and then incubated for 20 min at room temperature. After centrifugation, the supernatant was obtained and dried under nitrogen. The residue was dissolved in methanol and 10 μl was injected for LC-MS/MS analysis in multiple reaction monitoring (MRM) and in negative ion mode.
Results and Conclusions: Nineteen bile acids and two internal standards were detected within a twelve minute analysis time. For the healthy participants, the main bile acids detected were taurocholic (TCA), glycocholic (GCA), taurochenodeoxycholic (TCDCA), glycochenodeoxycholic (GCDCA) and glycoursodeoxycholic (GUDCA) acids. Bile acids profiling of serial specimen from two NEC patients were analyzed over time during their treatment, which included surgery and antibiotic treatment. Serum specimens from a patient suspected to have a peroxisomal disorder were analyzed as well. Further, age-related reference intervals will be established.

P-016

Discovery of novel biomarkers for mucopolysaccharide disorders in patient urine samples using label free proteomics

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Background: Development of novel treatments such as enzyme replacement therapies for Mucopolysaccharidoses means that quicker, better and more specific tests for diagnoses are required for patients with MPS. Current diagnosis of the Mucopolysaccharidoses includes the measurement of glycosaminoglycans in urine as a screening test and then further analysis of white cell enzymes. Most urine tests are still performed using 2days electrophoresis methodology which is semi-quantative and labour intensive.
Method: Label free proteomics was performed on urine samples from MPS I, II, VI and control.
Results: A total of 305 proteins were detected. Interestingly, 74 of these proteins were observed to be changed significantly ($p<0.05$) in the MPS groups compared to controls. Many of these novel markers were found to be involved with proteoglycan binding and extracellular matrix function. At least 6 proteins have never been reported previously in urine. Seven, ten and eight proteins were able to distinguish MPS I, II and VI from the other MPS disorders respectively.
Conclusion: Label free proteomics on urine from patients with MPS I, MPS II, MPS VI and controls was used to find new biomarkers and to produce a new test that could be used for diagnosis and monitoring disease progression/treatment.

P-017

Liquid-liquid extraction and solid phase extraction for urinary organic acids - a comparative study

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Introduction: The qualitative and quantitative analyses of urinary organic acid are important diagnostic tools for organic acidurias. The composition of urine samples may vary considerably and require sample preparation prior to GCMS analyses.
Materials and methods: We performed comparative studies on the isolation of organic acids from urine using liquid-liquid extraction and solid-phase extraction, the extracted residue was air dried, converted into trimethylsilyl derivatives and analysed by GC-MS.
Results: Here we present the % recovery of 16 organic acids by solvent and solid phase extraction. Lactate – 72.75/67.8, oxalate – 113.25/78.9, pyruvate – 81.75/108.75, methylmalonate – 99/99.6, ethylmalonate – 83/87.25, fumarate – 75.25/96.25, lactate dimer – 98.5/60, glutarate – 100.5/99.6, 3-methylglutarate – 82.5/80.4, 3-phenylbutyrate – 84.75/64.6, adipate – 83.25/201, suberate – 105.75/211, azelate – 133.5/97.9, sebacic acid – 98.25/93.4, orotate – 56/85.75, succinylacetone - 67.75/123.4. In SPE phosphate and urea are the major unwanted peaks that mask the other metabolites in the