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PRENATAL DIAGNOSIS OF ORGANIC ACID METABOLIC DISORDERS WITH AMNIOTIC FLUID BY ELECTROSPRAY TANDEM MASS SPECTROMETRY

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A rapid electrospray tandem mass spectrometry (ESI-MS/MS) method was studied for the prenatal diagnosis of organic acidemia based on the acylcarnitines levels in amniotic fluid. The acylcarnitines were derivatized with butanolic hydrogen chloride and quantified using the corresponding stable isotopes as internal standards. Mass spectrometric data of the butylester derivatives of acetyl-, propionyl-, butyryl-, isovaleryl-, and octanoyl-carnitine were acquired by product ion scanning with product ion at m/z 85, which was common to the butylester of acylcarnitines. The recoveries for acylcarnitines were between 93 and 98% and the coefficient of variations were around 9.0%. The reference ranges for acetylcarnitine and propionylcarnitine were determined to be 2.05±0.54 nmol/ml (mean±SD, n=29) and 0.75±0.35 nmol/ml, respectively. Amniotic fluid samples collected from high risk pregnancies for propionic acidemia were investigated by this method. One case with elevated propionylcarnitine/acetylcarnitine ratio (1.22) was diagnosed as propionic acidemia as compared to the ratio for controls (0.37¡Ó0.11, n=29). The case was confirmed to be propionic acidemia by *in vivo* propionic acid incorporation assay. By using the same scanning mode with product ion at m/z at 85, this quantitative assay of acylcarnitines in amniotic fluids by ESI-MS/MS could also be a useful tool for prenatal diagnosis of methylmalonic acidemia and other organic acidemia.

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DIAGNOSING DISORDERS OF MITOCHONDRIAL FATTY ACID OXIDATION: MEASURING SERUM 3-HYDROXY-FREE FATTY ACIDS (3-OHFFAS) BY STABLE ISOTOPE DILUTION GC-MS.

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Disorders of fatty acid oxidation are difficult to diagnose, primarily because associated metabolic intermediates are generally only seen during acute illness. This study describes a serum assay for the measurement of 3-OHFFA's. Elevated levels of these metabolites should be indicative of disorders of the 3-OH-acyl CoA dehydrogenases, LCHAD and SCHAD. The assay utilizes GC-MS stable isotope dilution to measure the 3-OHFFAs with carbon chain lengths from C6 to C16. Stable isotopes of these six compounds were synthesized with ^{13}C in positions 1 and 2 of the 3-OH-FFA. The assay is linear in the range of 0.2 to 50 uM for all six 3-OHFFAs and shows good precision at all levels measured, i.e. intra-assay CVs range from 1.5 to 7.9% and inter-assay CVs range from 1.0 to 15.8%. Recoveries range from 87 to 114% for the six compounds at two different concentrations. Reference ranges in umol/L for the six 3-OHFFAs were established using 43 pediatric and adult individuals with no defects in fatty acid oxidation, and are as follows: 3-OH-C6: 0.4 - 2.2 , 3-OH-C8: 0.2 - 0.9, 3-OH-C10: 0.2 - 0.5, 3-OH-C12: 0.2 - 0.4, 3-OH-C14: <0.3 and 3-OH-C16: <0.3. Infants receiving formula containing medium chain triglycerides (MCT) showed elevated 3-OHFFAs. No other interferences were found. Two patients diagnosed with LCHAD deficiencies show elevations in all the 3-OHFFAs, with marked elevations in 3-OH-C14 and 3-OH-C16 concentrations. Two patients diagnosed with SCHAD deficiencies show elevated 3-OH-C6, 3-OH-C8 and 3-OH-C10, but only mild to no elevations in the longer carbon chain length 3-OHFFAs (C12 to C16).

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ON THE PRECISION, ACCURACY AND LINEARITY OF THE RADIOMETER EML™ 105 WHOLE BLOOD METABOLITE BIOSENSORS

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The analytical performance of the whole blood glucose and lactate electrode system of the EML™ 105 analyzer (Radiometer Medical A/S, Copenhagen, Denmark) was evaluated. During the evaluation the instruments were used in daily routine, measuring approximately 80 pediatric heparinized whole blood capillaries per analyzer per day.
For glucose, between-day CV's were ≤ 1.9%, irrespectively of the concentration; for lactate, between-day CV's were ≤ 3.1%. Using either aqueous or protein-based standards, glucose recoveries were 100 ± 10% whereas lactate recoveries depended on the matrix of the standards. Lactate was systematically underestimated in aqueous standards (~ -10%). In standards containing 40 g/L albumin recoveries were highly overestimated at low concentrations and declined steadily with increasing lactate levels but were within Radiometer performance specifications between 0.94 and 30 mmol/L. Carry-over was investigated according to NCCLS EP10-T2 and could be excluded (P ≤ 0.01). Glucose and lactate biosensors equipped with new membranes were linear up to 60 and 30 mmol/L, respectively but linearity fell upon daily use and increasing membrane lifetime. In conclusion, the Radiometer metabolite biosensors produce reproducible and accurate results in the normal measuring range. However, protein-based standards with high glucose and lactate concentrations should be introduced to monitor the time-dependent biosensor performance.

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STANDARDIZATION OF TOTAL BILIRUBIN MEASUREMENTS IN NEONATES

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A project for improvement of total bilirubin measurements at clinically significant ranges for neonates was carried out. This was accomplished by an original evaluation of the status Quo , preparation and provision of bilirubin calibration material and finally standardization of the method. A standard was prepared as per Doumas with 40 mg%. Dilutions of this stock were made and provided to selected neonatal hospital laboratories. The intraassay variations and the bias were obtained under the existing conditions. The Methanol method was found to be still widely used due to ease of technique and also being more economical. The C.V.'s and the bias were found to be of the order of 8% and 20% respectively. A standard with 20mg% concentration accompanied with Boehringer Mannheim controls were then distributed through the labs. Their techniques were also substituted with Jendrassik Groff WHO recommended procedure. The trial was repeated the bias was found to have decreased to about 1% and the CV's to 4%. It is concluded that despite the reluctance of laboratories to switch over to the new method due to aforementioned reasons ,valid measurement of bilirubin in neonates can only be achieved by employing a method validated to at least 20 mg% and simultaneous use of bilirubin standard with high concentrations.