Supplement 6. Study characteristics of included studies

| **Study reference** | **Country, time period** | **Study design** | **Source and type of material** | **Age at specimen collection** | **Method of extraction and derivatisation** | **Type of TMS and conditions** | **Analytes and cut-off** | **Re-testing of positive samples and reference standard** |
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| **ASIA** |
| Mak 2018  | Hong Kong1 October 2012-31 August 2014 | Pilot screening study in 2,440 neonates. 3 public hospitals and the University of Hong Kong | Heel prick test in compliance with Clinical and Laboratory Standard Institute Guidance. | 24h-28 days*(24 - 48h n=2064 [84.6%],* *3 - 5 days n=331 [13.6%],* *5 - 7 days n=9 [0.4%],* *7 - 28 days n=36 [1.5%]).* | Two commercial DBS assay kits: (1) MassChrom® Amino Acids and Acylcarnitines from Dried Blood/Non Derivatised (Chromsystems Instruments & Chemicals GmbH, Germany); and (2) NeoBaseTM Non-derivatized MSMS kit (PerkinElmer, USA). Fully automated online extraction system(DBS-MS 500; CAMAG, Muttenz, Switzerland).(1) and (2) No derivatisation. |  LC-TMS.Acylcarnitines: MRM. No further details provided | Not specified which markers were used for LCHADD/MTPD | Positive samples reanalysed. Reference standard: measurements of functional metabolites (mainly plasma acylcarnitine levels, and urine organic acid levels) and genetic diagnosis by DNA sequencing wherever appropriate.  |
| Yang 2018  | China2014 – 2015 (1 year) | Propsective newborn screening study in 100,077 neonates.Jining city.Number of centres not reported | DBS on Whatman 903 paper. | 3 to 37 days | NeoBaseTM Non-derivatized MSMS kit (Perkin Elmer, MA, USA).No derivatisation. | Triple quadrupole TMS equipped with an ACQUITY TQ detector (Waters, MA, USA) coupled to a Waters 1525 binary HPLC pump.MRM mode. | LCHADD: C16OH>0.04 µmol/LMTPD: C18OH >0.03 µmol/LC18:1OH >0.05 µmol/L | TMS test repeated and compared to first results. Urinary organic acids determined by GC-MS, or high precision DNA mass spectrometry for 39 genetic mutations. |
| **EUROPE** |
| Bonham 2014 | United KingdomJuly 2012 – July 2013 (1 year) | Prospective expanded newborn screening study in 436,969 neonates.6 screening laboratories. | DBS on Guthrie filter paper. | 5-8 days as per UK regulations | Extraction not reported.Underivatised eluates. | MRM TMS *m/z* 416 to 82 transition.  | Analytical cut-off: C16OH>0.12 μmol/L.Screening cut-off:C16OH>0.15 μmol/L – lowered from 0.20 μmol/L following a missed case.C16:1OH; C18OH.Cut-off NR. | If analytical cutoff for C16OH positive, assay C16OH, C16:1OH andC18OH in duplicate (using fresh disks) and use screening cutoff.Referred directly to specialist metabolic physician.Reference standard: blood acyl carnitines, urine organic acids, blood DNA G1528C mutation analysis, and enzyme assay if needed. |
| Couce 2011 | Galicia, SpainJuly 2000 – July 2010 (10 years) | Prospective newborn screening study in 210,165 neonates. | DBS on Whatman 903 paper. | Up to 2002:5-8 days.2003-2010: on day 3. | NR | MS/MS Applied Biosystems Sciex 2000 apparatus | C16OH,C18:1OH, C18OHCut-offs not reported | When a positive result was found in the DBS sample, repeat or additional analyses were performed on the urine samples.Clearly aberrant results immediately referred to clinical unit.For borderline results, a second sample was requested by NBS lab. If second result also positive, the patient was referred to clinical unit.Reference test: Enzyme and/or molecular studies.  |
| Lindner 2011Schulze 2003  | South-West Germany April 1998- September 2001 {Schulze 2003}January 1999 - June 2009(10 years){Lindner 2011} | Prospective newborn screening programme in 250,000 neonates up to 2001. Overlap with 1,084,195 screend newborns reported by Lindner et al. (2011).1 screening laboratory. | DBS on Schleicher & Schuell 2992 up to 1999, then on Schleicher & Schüll 903 since 2000.  | Up to 2001:Recommended time between 3rd – 7th days of life. Median 5th day. {Schulze 2003} Up to 2005: Recommended between day 3-5.2005 onwards:36-72 hours.{Lindner 2011} | Extraction using methanol stock solution of internal deuterated standards (0.04 µmol/L [2H9]myristoylcarnitine [C14], 0.08 µmol/L [2H3]palmitoylcarnitine [C16]).Derivatisation to butyl esters.When a specific stable isotope was not available, the following ratios were used for calculation: C14OH/C14-d9, C16:1/C16-d3, C16:1OH/C16-d3, C16OH/C16-d3, C18:1/C16-d3, C18/C16-d3, and C18:1OH/C16-d3. | API 365 triple quadruple TMS with an ion spray device.Acylcarnitines measured by positive precursor ion scan of *m/z* 85 (scan range *m/z*: 200–500). | C14OH (>0.12 µmol/L) and/or C16:1OH (>0.22 µmol/L); C16OH (>0.20 µmol/L); C18:1OH (>0.12 µmol/L); C18OH (>0.11 µmol/L).Cut-off prespecified: 99.5th percentile based on data collected from 10,000 healthy neonates. | Repeat analysis from the same blood spot: only TP if both samples positive. If a distinct discrepancy, a third test was done and the mean of the 2 corresponding results was used and interpreted by an experienced metabolic disease specialist.Presumptive positive cases repeated DBS test on recall or referral to a treatment centre. Reference standard: Enzyme activity in fibroblasts/lymphocytes {Schulze 2003} – acylcarnitine profile in plasma/DBS and/or genotype and/or enzyme activity {Lindner 2011}. |
| Lund 2012  | Denmark, Faroe Islands and GreenlandTrial period: February 2002 – February 2009 (7 years).Routine expanded screening: February 2009 – March 2011. | Prospective pilot and routine expanded screening programme in 504,049 newborns.1 screening laboratory. | DBS on Schleicher & Schüll 903 filter paper up to 2010, then replaced by Ahlstrom 226 filter paper. | Trial period:Recommended age 4-9 days after birth (median 5 days). From February 2009:Recommended age 2-3 days; median age 2.5 days. | February 1st, 2002, to June 30th, 2003: Analysis using a protocol developed at Statens Serum Institut, Copenhagen based on analysis of relevant acylcarnitine butyl esters.From July 1st, 2003,until February 2nd, 2009:PerkinElmer NeoGram Amino acids and acylcarnitines tandem mass spectrometry kit™ (MS-8970EY).Derivatisation to butyl esters.From February 2nd, 2009: PerkinElmer NeoBase non-derivatized MSMS kit™ (3040-0010). No derivatisation. | SciEx API2000 up to 30th June 2003; acylcarnitines as precursors of *m/z* 85.TMS conditions unclear for time period July 2003-February 2009.From 2nd February 2009:Waters Micromass Quattro micro™ tandem massspectrometers. | Primary: C16OH >0.12U;Secondary: C18:1OH >0.1U.Cut-off pre-specified. | Positive samples reanalaysed in duplicates. If abnormal profiles confirmed, the Centre for IMD Copenhagen University Hospital contacted for confirmatory tests. Reference standard: Urine organic acids, plasma acylcarnitines, molecular-genetic analyses. |
| Sander 2005  | Germany1999 – 2005(6 years) | Prospective screening programme in 1,200,000 neonates. 1 screening laboratory. | DBS on S&S 2992 filter paper (Schleicher & Schüll).  | Recommended age:36-72 hours.97.5% by day 5. Some several days later.  | Extraction using methanol containing deuterated internal standards (D3-C14, D3-C16 and D3-C18) and derivatisation to butyl esters. | 3 TMS (TMS quatro LC, Micromass, Manchester). Long-chain hydroxyacylcarnitines measured in MRM mode; remaining acylcarnitines and free carnitine in full scan MCA mode. | C14:1 >0.35 µmol/L;C14OH >0.2 µmol/L;C16OH >0.08 µmol/L;C18:1OH >0.06 µmol/LThreshold chosen after evaluation of data from patients with known MTPD and 5,000 normal controls.  | Repeated analyses of fresh dried blood spots.Reference standard: Enzyme analysis in cultured fibroblasts or lymphocytes, mutation analysis. |
| Smon 2018  | Slovenia2013-2014 | Retrospective pilot study of blood spots in 10,048 newborns. | DBS. | 48-72 hours.Analysed after 6-11 months storage (unclear how stored). | Chromsystems’ kit “Amino Acids and Acylcarnitines from Dried Blood” (Graefelfing, Germany):Analytes were extracted using the extraction buffer with added internal standards. Derivatisation to butyl esters. | PerkinElmer 200 HPLC system coupled to AB Sciex 3200 QTRAP (AB Sciex, Singapore). | C16:1OH, C16OH, C18:1OH, C16OH/C16Cut-offs set at 99.9th percentiles after the completion of the study. | Children with the highest probability of IEM were invited for an outpatient visit at the University Children’s Hospital for follow-up tests.Reference standard: Next-generation sequencing, organic acids in urine, additional acylcarnitine test using DBS. |
| **USA**  |
| Frazier 2006  | North Carolina, USAJuly 1997 – July 2005(8 years). | Prospective newborn screening programme in 944,078 neonates.2 screening laboratories. | DBS on Schleicher & Schüll 903 filter paper. | At least 24 hours regardless of gestational age and feeding status. Mean age 39 hours. | July 1997 - April 1999:Performed at Neo Gen laboratory using commercial testing.From April 1999: In-house extraction method by Millington et al. (1990) {REF}:Extraction using methanol with deuterated internal standards. Derivatisation to butyl esters. | Micromass Ltd/Water Corp Quattro LC TMSGilson instruments 215 Liquid Handler autosampler and Hewlett Packard/Agilent Technologies series 1100 Isocratic HPLC pump. | Since January 2003:C16OH >0.18 µmol/L (single cut-off);C18:1 >4.08 µmol/L (independent elevation not diagnostic), C18:1OH >0.14 µmol/L (independent elevation not diagnostic).Cut-offs initially set at ~4 SD above the mean of results obtained from 2,000 sequential newborn samples. Cut-offs were modified over time to minimise FP and FN.  | Repeat analysis from another disk punched from the same card.If results were borderline a request was sent for a repeat sample. If above the diagnostic cut-off referal to a metabolic specialist.Reference standard: Urine organic acids and a plasma acylcarnitine profile. Enzyme and mutation analyses done whenever the tests were available and approved by 3rd party reimbursers. |
| Zytkovicz 2001  | New England Newborn Screening Program (NENP) testing newborns in Massachusetts, but also from Maine, New Hampshire, Vermont, and Rhode Island (USA).1999-2001(2 years) | Prospective screening programme in 257,000 neonates.Only 164,000 Massachusetts newborns were screened for LCHADD.1 screening laboratory. | DBS on S&S Grade 903 filter paper (Schleicher & Schüll). | Most DBS obtained 1-3 days after birth. | In-house method:Extraction using methanolic internal standard solution ([2H3]-palmitoylcarnitine([2H3]-C16), 0.06 µmol/L).Derivatisation to butyl estesr. | 1100 Hewlett Packard HPLC pump. Model 215 Gilson autosampler sent sample to MS/MS.Micromass Quattro LC triple-quadrupole TMS.Analyzed with MRM mode; for acylcarnitines (35–54 V, 25 eV) ion abundance of the fragment ions was determined at *m/z* 85. | C16OH/d-C16Cut-off: 0.1.Cut-off set at 7 SD above population mean such that ~0.02% or less of population of ~4,000 random newborns would be flagged for each marker. | Repeat analysis from another disk punched from the same card. Mean of the 2 tests was calculated, and if it was greater than the flag value, results were reported to follow up.If one of more markers were out of range a full metabolic work up was recommended. If initial specimen had very increased marker then full metabolic work up initiated straight away.Reference standard: According to standard metabolic criteria. |

DBS, dried blood spots; DNA, deoxyribonucleic acid; FN, false negative; FP, false positive; GC, gas chromatography; HPLC, high-performance liquid chromatography; IEM, inborn errors of metabolism; IMD, inherited metabolic disease; LC, liquid chromatography; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MCA, multi-channel acquisition; MRM, multiple reaction monitoring; MTPD, mitochondrial trifunctional protein deficiency; NBS, newborn blood spot screening; NR, not reported; SD, standard deviation; TMS, tandem mass spectrometry; TP, true positive.