

NEWBORN SCREENING: CLINICAL ADVANTAGES OF LCMSMS

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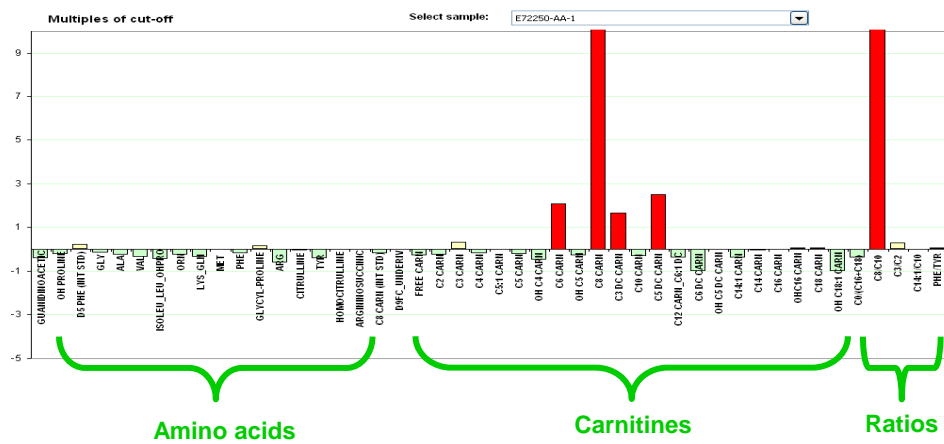
Introduction of MSMS to newborn screening in Australasia

- From 1998, NBS labs started to introduce MSMS for several inborn errors of metabolism
- Dried blood spots extracted with methanol containing ²H or ¹³C internal standard
- +/- derivatisation
- Electrospray quadrupole MSMS
- Targeted panel of metabolites (amino acids and acyl carnitines) using multiple reaction monitoring
- Direct injection so actually no LC, ~1.5 min run time
- Metabolites are quantitated against internal standards (CV's ~ 10-20%)
- Multiplexing: one test:many biomarkers (38):many disorders (24)

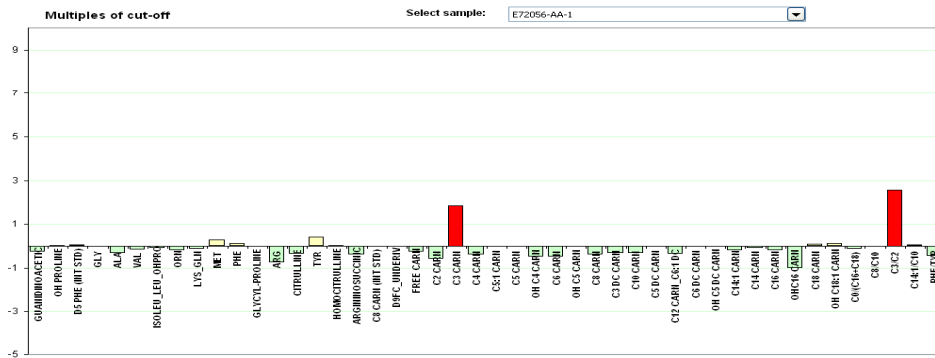
How many disorders are detected by tandem mass spectrometry?

Disorder(s)	Other names	Incidence	Marker	Genes involved
List A: Serious conditions, generally good sensitivity				
1. Phenylketonuria including BH ₄ defects	PKU	1:14,000	Phe, Phe/Tyr	PAH, DHPR, GCH1, PTS, PCBD
2. Medium chain acyl CoA dehydrogenase	MCAD	1:12,000	C8 carn	ACADM
3. Glutaric acidaemia type 1	GA1	1:90,000	C5DCcarn	GCDH
4. Very long chain acyl CoA dehydrogenase	VLCAD	1:40,000	C14:1carn	ACADVL
5. Carnitine uptake defect	CUD	1:68,000	↓C0 carn	SLC22A5
6. Propionic acidaemia etc.....	PA	1:140,000	C3 carn	PCCA, PCCB
24. Holocarboxylase synthase	HCS	1:500,000	OHC5 carn	HCS
List B: Less serious or asymptomatic conditions that can be detected				
1. 3-methyl crotonyl CoA carboxylase	3MCCC	1:80,000	OHC5 carn	MCCA, MCCB
2. Short chain acyl CoA dehydrogenase etc	SCAD	1:50,000	C4 carn	ACADS
List C: Serious conditions, poor sensitivity				
1. Tyrosinaemia type 1	TYR1	1:140,000	Tyr	FAH
2. Ornithine transcarbamylase etc.....	OTC	1:80,000	↓ Cit	OTC
List D: Maternal conditions				
1. B12 deficiency			C3 carn	
2. 3MCCC, CUD, GA1, MCAD				

Newborn screening MSMS panel example (a)



Newborn screening MSMS panel example (b)



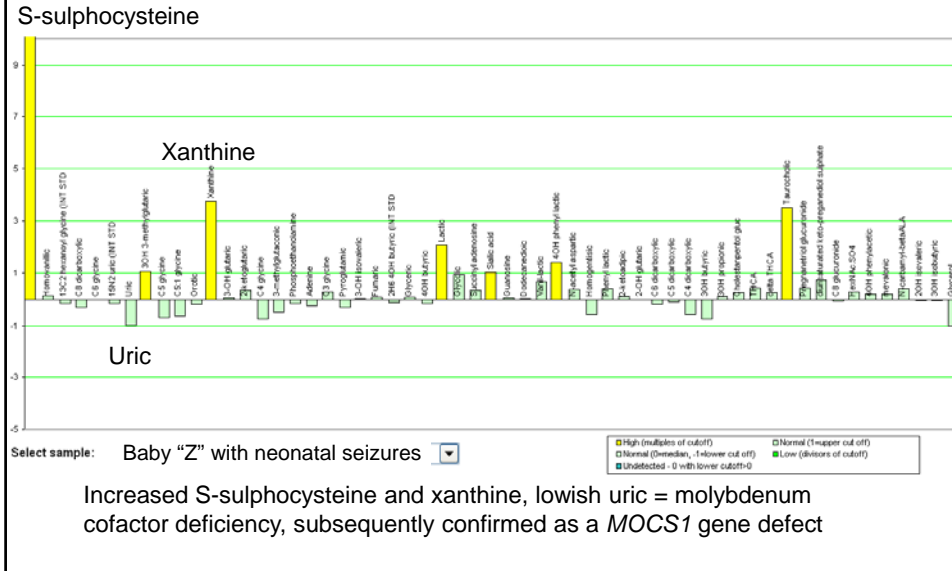
- ↑C3, supported by ↑C3/C2 ratio
- ? false positive, maternal B12 deficiency, methylmalonic acidaemias, cobalamin defects, propionic acidaemia

Confirmation of NBS screen positive cases

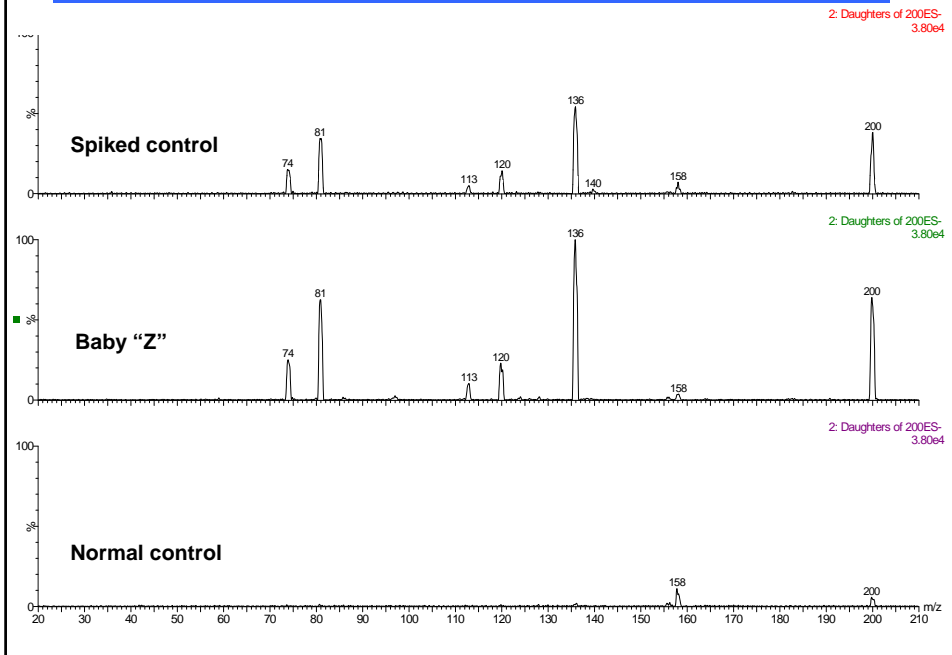
- Newborn screening is not diagnostic
- Confirmatory methods: ideally test an independent marker in an independent sample e.g.

Disorder	Primary NBS marker	Biochemical confirmatory test
MCAD	C8 carnitine	Urine hexanoyl glycine
Methyl malonic acidaemia	C3 carnitine	Urine or plasma methyl malonate
Propionic acidaemia	C3 carnitine	Urine methyl citrate
Cobalamin disorders, B12 deficiency	C3 carnitine	Urine or plasma MMA, plasma total homocysteine

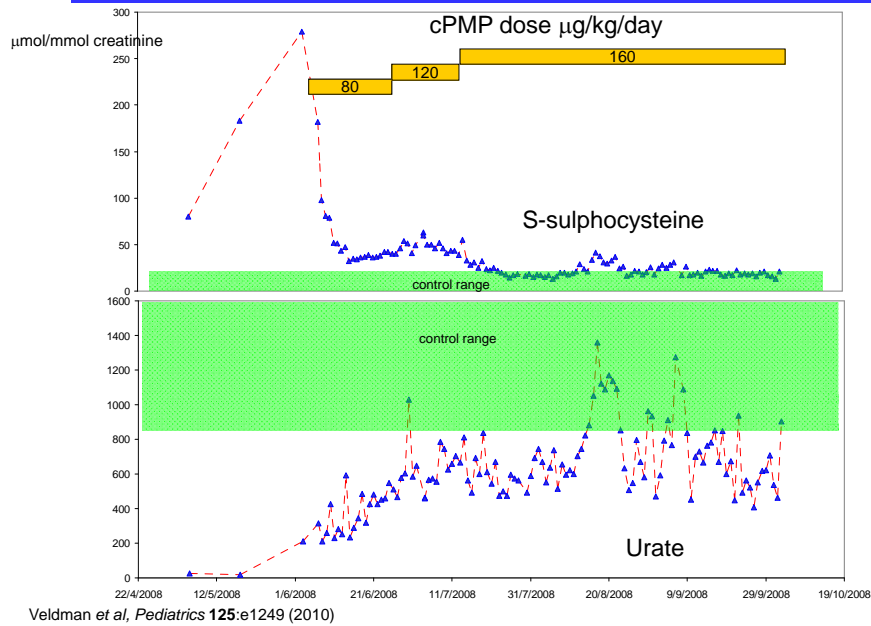
Urine screening: detecting other neonatal disorders negative ion mode



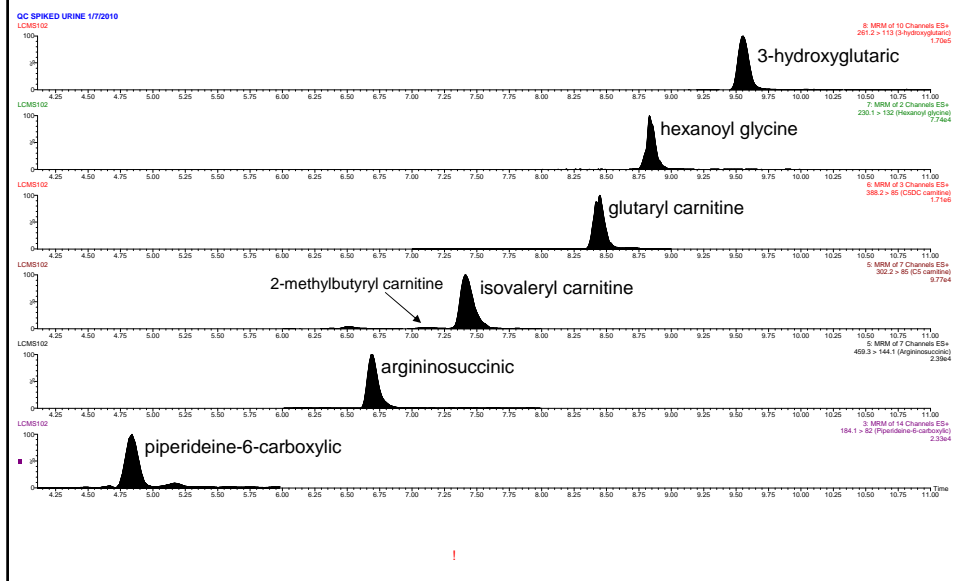
Confirmation of S-sulphocysteine with product ion spectra



Monitoring urine metabolite levels in baby "Z" First MOCS1 patient treated with cPMP cofactor replacement therapy



Quantitative multi analyte LC-MSMS urine and dried blood spots



Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study

Wilcken *et al*, *Lancet* 369(9555):37 (2007)

Findings

In cohorts aged at least 4 years there were 35 MCAD-deficient patients in those not screened (2.28 per 100 000 total population) and 24 in the screened population (5.2 per 100 000). We estimated that patients with this disorder in the unscreened cohort remained undiagnosed. Before 4 years of age, three screened patients had an episode of severe decompensation (including one neonatal death) versus 23 unscreened patients (including five deaths). At the most conservative estimate, relative risk of an adverse event was 0.44 (95% CI 0.13–1.45). In the larger cohort the relative risk (screened vs unscreened) of an adverse event by age 2 years was 0.26 (95% CI 0.07–0.97), also a conservative estimate. 38 of 52 living patients had neuropsychological testing, with no suggestions of significant differences in general cognitive outcome between the groups.

Interpretation

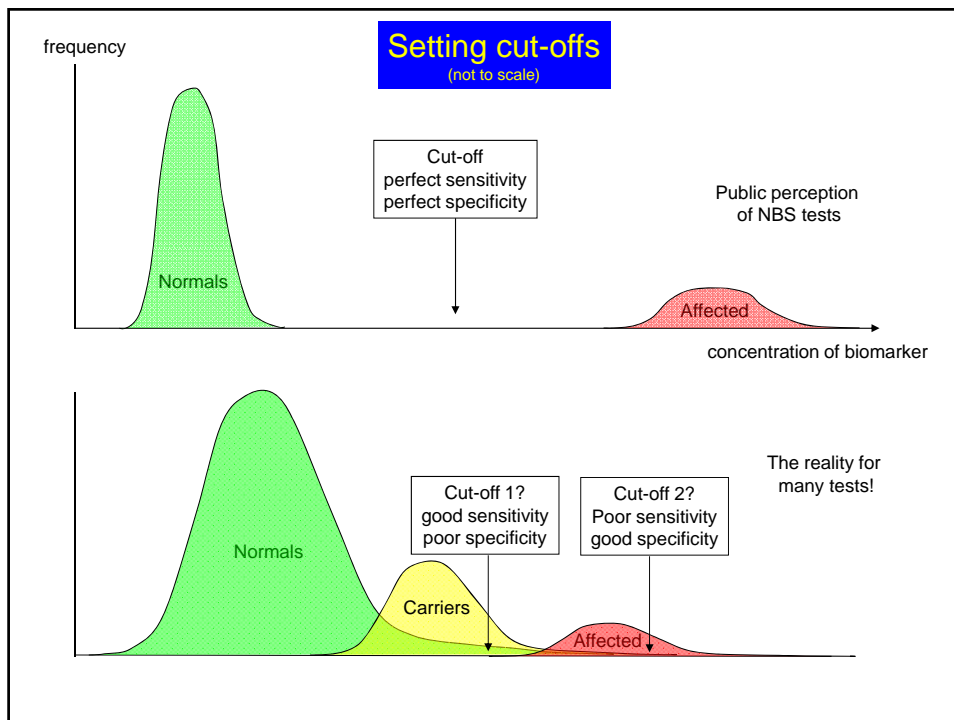
Screening is effective in patients with MCAD deficiency since early diagnosis reduces deaths and severe adverse events in children up to the age of 4 years.

Expanded Newborn Screening: Outcome in Screened and Unscreened Patients at Age 6 Years

Wilcken *et al*, *Pediatrics* 124:e241 (2009)

RESULTS: Inborn errors, excluding phenylketonuria, were diagnosed in 116 of 1 551 200 unscreened infants (7.5/100 000 births) and 70 of 461 500 screened infants (15.2/100 000 births). Excluding MCADD, 21 unscreened patients with metabolic disorders diagnosed after 5 days of life died or had a significant intellectual or physical handicap (1.35/100 000 population) compared with 2 of the screened cohort (0.43/100 000; odds ratio: 3.1 [95% CI: 0.73–13.32]). Considering the likely morbidity or mortality among the expected number of never-diagnosed unscreened patients, there would be a significant difference. Growth distribution was normal in all cohorts.

CONCLUSION: Screening by tandem mass spectrometry provides a better outcome for patients at 6 years of age, with fewer deaths and fewer clinically significant disabilities. *Pediatrics* 2009;124:e241–e248



Setting the cut-offs

False positives:

- cause parental stress
- extra work for lab and follow-up team
- too many lead to complacency in follow-up (“This always turns out to be normal”)
- Want to keep overall false positive rate <0.5%

False negatives:

- erode confidence in screen

Easy to define reference range, not so easy to define disease range for rare disorders

⇒ inter laboratory collaboration required



Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: A worldwide collaborative project

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McHugh et al, *Genetics in Medicine* 13:230 (2011)

Second-tier testing

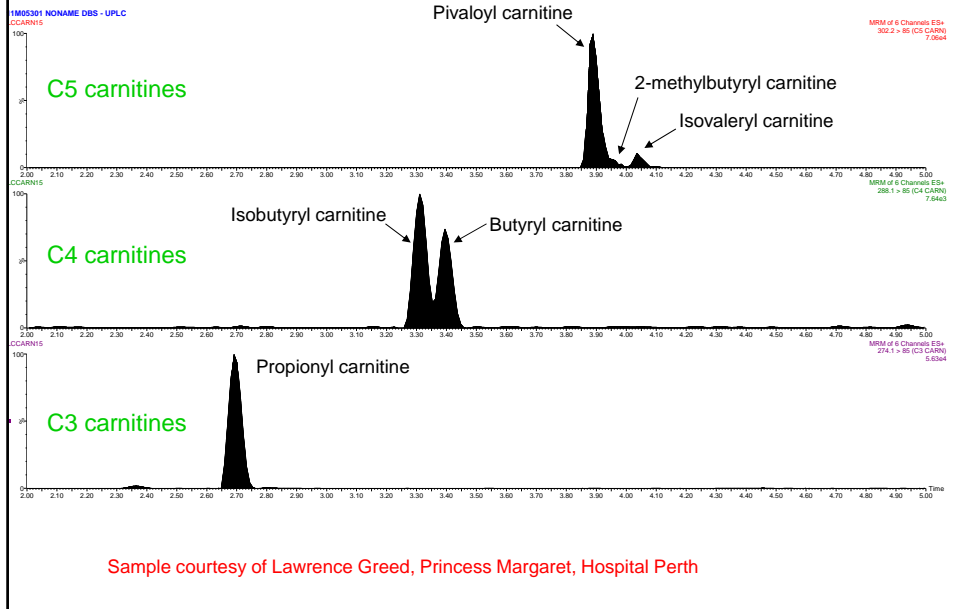
- Some markers still give a poor PPV despite a lot of data/collaboration/harmonisation on cut-offs
- This could be due to analytical limitations in the screening test (e.g. interferences, poor CV) or maybe the marker is not that good
- A second-tier test on the original sample can be used to improve the overall screening performance
- This could involve improving the analytical performance or (better) measuring another independent marker
- LC-MSMS is often used

Some second-tier NBS LC-MSMS tests

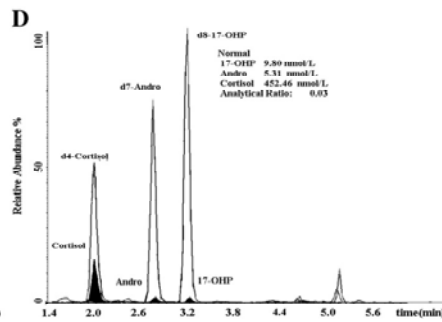
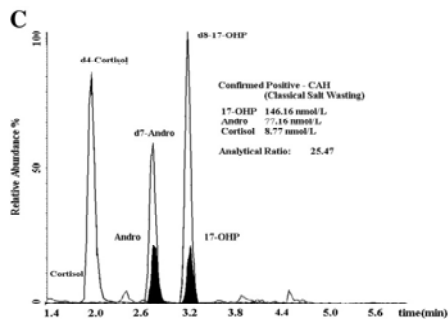
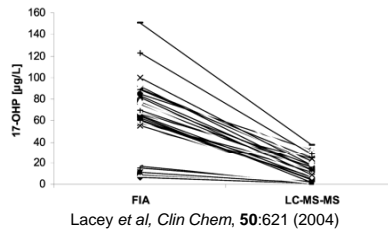
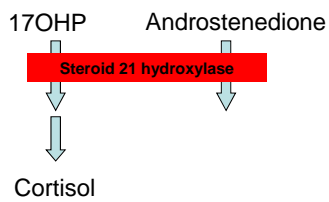
analysis of original dried blood spot sample

First tier test	Second tier test	Target disorder
Tyrosine	Succinyl acetone	Tyrosinaemia type 1
Xleucine	Allo-isoleucine	Maple syrup urine disease
C3 carnitine	Methyl malonic Total homocysteine Methyl citrate	Methyl malonic acidaemia, B12 def Cobalamin defects, B12 def Propionic acidaemia
Methionine	Total homocysteine	Homocystinuria
C5 carnitine	Isovaleryl carnitine 2-methylbutyryl carnitine Pivaloyl carnitine	Isovaleric acidaemia SBCAD Antibiotic interference

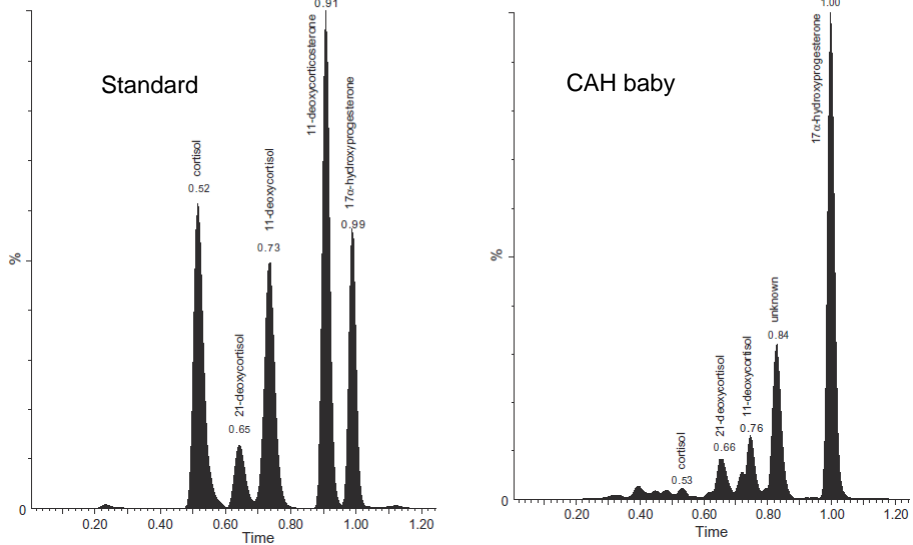
UPLC-MSMS separation of dried blood spot acyl carnitines



LC-MSMS second tier testing for congenital adrenal hyperplasia



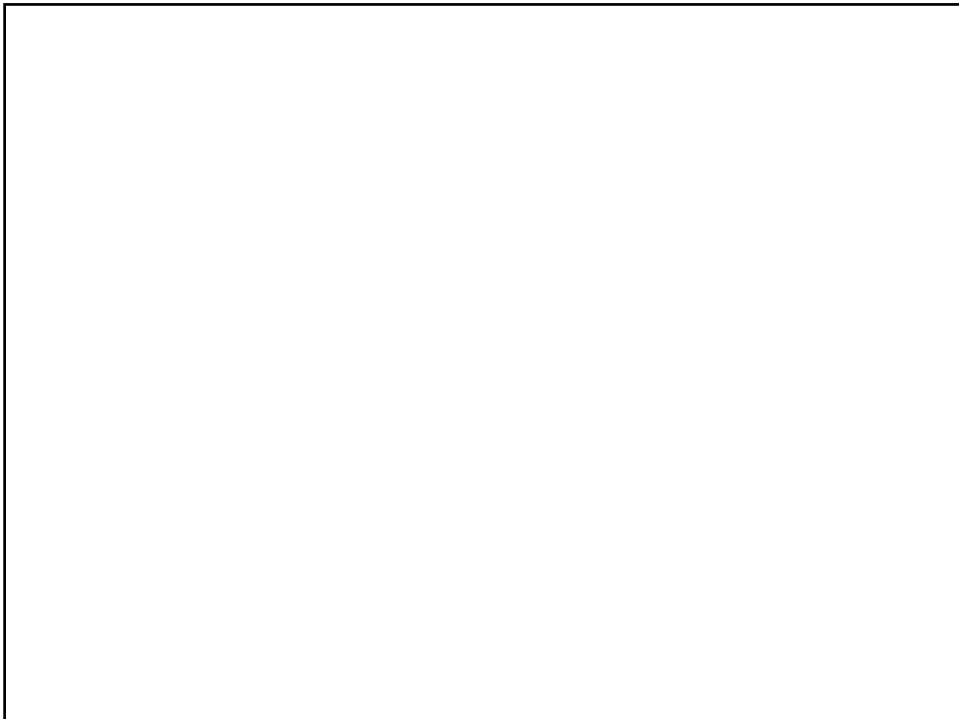
UPLC-MSMS second tier testing for congenital adrenal hyperplasia



? suitable as a first tier test

Janzen *et al*, *Steroids* (in press 2011)





Abstract

NEWBORN SCREENING: CLINICAL ADVANTAGES OF LCMS

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From ~2000, newborn screening laboratories throughout Australia, Europe and North America have introduced tandem mass spectrometry screening for ~25 inborn errors of metabolism using a panel of amino acids and acyl carnitines. Cycle times of ~1.5 mins can be achieved with direct injection, allowing newborn screening batches to be run unattended overnight. False positives rates <0.5% are readily achievable and international collaborative projects are helping to reduce rates further. Clear benefits of earlier diagnosis in terms of reduced morbidity and mortality have been demonstrated for some of the more prevalent disorders such as medium chain acyl CoA dehydrogenase deficiency. A useful side benefit of expanded newborn screening is the diagnosis of maternal B12 and carnitine deficiencies. Chromatographic methods are not easily implemented as a first-tier newborn screening test but can be used as a second-tier test to improve the overall sensitivity and/or specificity of newborn screening. Examples include measurement of total homocysteine, methylcitric and methylmalonic acids for the investigation of samples with increased propionyl carnitine. Newborn screening immunoassays for 17-hydroxyprogesterone have a high false positive rate due to interfering steroids in premature babies and LCMS measurement of 17-hydroxyprogesterone and other steroids is an important second-tier test to improve this situation. LCMS technology also plays an important role in the rapid confirmation and classification of screen positive cases through urine analysis.