



a place of mind

THE UNIVERSITY OF BRITISH COLUMBIA

# Integrated Second-tier MSUD testing by LC-MS/MS

**Graham Sinclair, PhD FCCMG**

Newborn Screening Laboratory

BC Children's Hospital

University of British Columbia

Vancouver, BC, Canada



# Benefit vs. Cost

- Large benefit to few
  - Clear treatment benefit
  - Avoidance of severe acute events
  - Less uncertainty for families



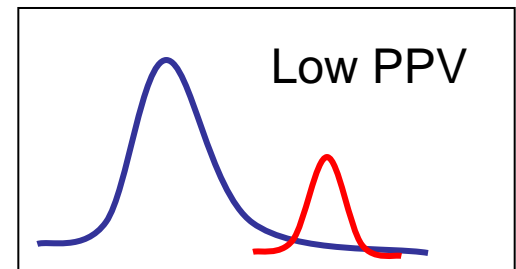
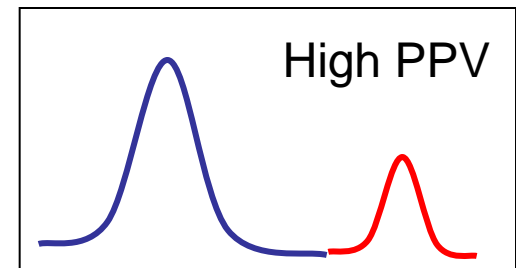
- Small cost to many
  - False positives
  - Non-disease states
  - Financial costs

# Test Performance

- Target PPV = 20% overall

- For a sensitivity >99%

- PKU = 80% PPV
- MCAD = 60% PPV
- PA/MMA = **1.5%** PPV
- CAH = **0.6%** PPV
- MSUD = **0.2%** PPV



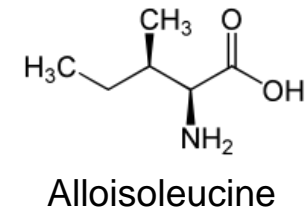
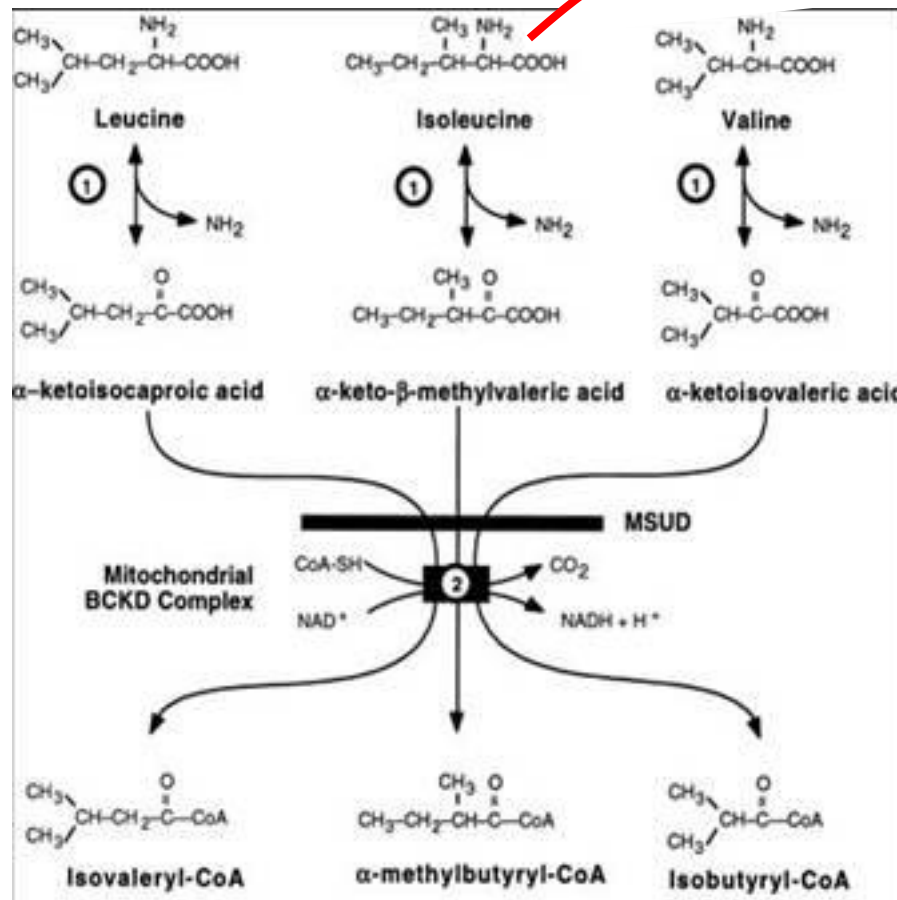
- Two-tier testing can help to minimize false positives and increase PPV.

# Maple Syrup Urine Disease (MSUD)

M/Z = 131.1

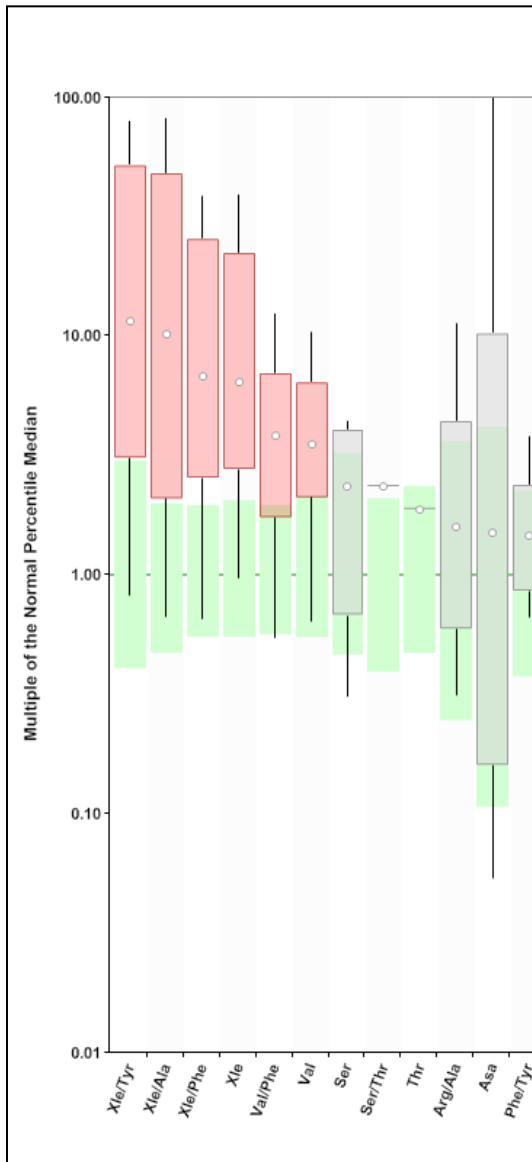
M/Z = 131.1

M/Z = 131.1





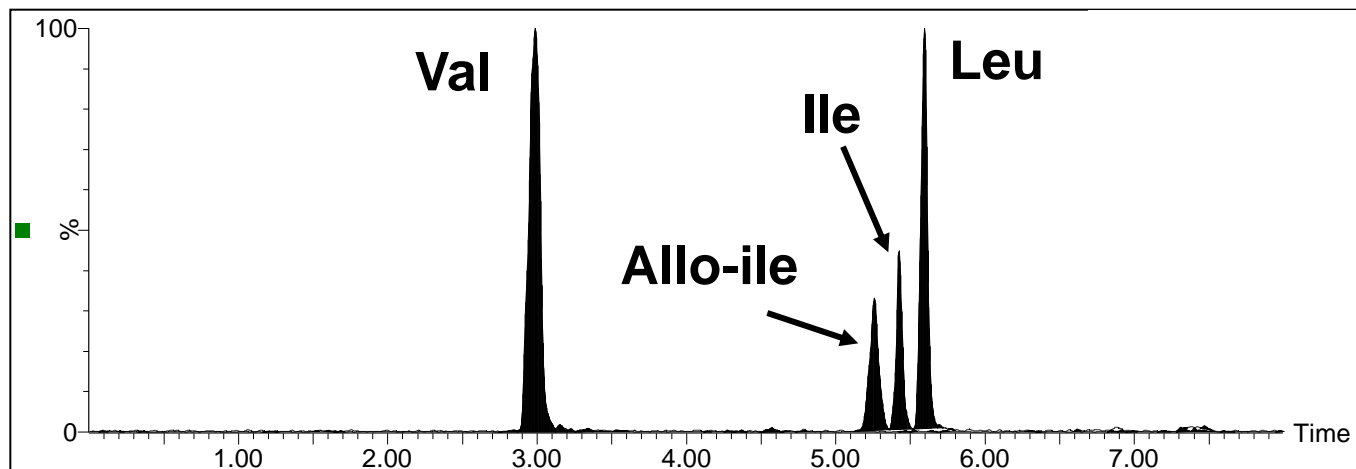
# MSUD Single Tier Algorithm



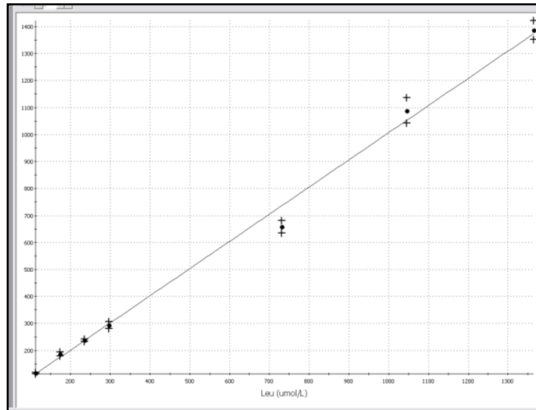
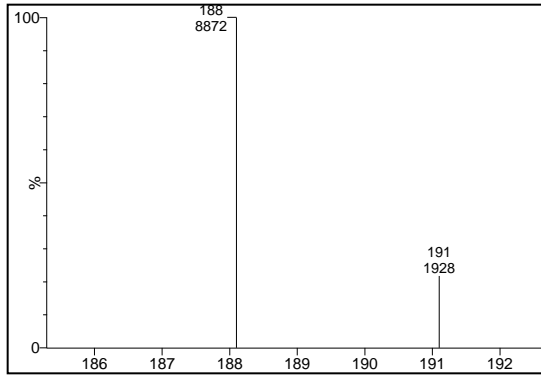
- Xle AND Val AND (Xle/Phe OR Val/Phe)
- Cutoff at 85% individual sens.
- Predicted:
  - 2FP/year in BC
  - Incidence 1/100,00-1/200,00
  - 1 case every 2-3 years
- Actual (1 year of screening):
  - 2FP, no cases (?PPV)
- What is the true sensitivity?

# MSUD Second-Tier

- Chromatography to separate isomers
- 10 minutes per sample
- Allo-isoleucine specific for MSUD
- Requires repunch of the original card
- Oglesbee et al. 2008



# First-Tier MS/MS Calibration



- Most Programs:
  - Analyte ratio to ISTD
  - Correction by fixed response factor
- BCCH Method:
  - 7 point calibration curve
  - Analyte ratio to ISTD
  - correction by calibration curve
- Interday CV ~15% lower
- Better estimate of recovery
  - CDC QC regression slopes



Dieses Feld mit den Daten der Mutter ausfüllen

Name, Vorname des Versicherten  
**Mother's Name, Forename**  
 Address, Date of birth

Kassen-Nr. Versicherten-Nr. Status  
 Betriebsstellen-Nr. MZ-Nr. Datum

Lebzeiten-Grund  
 verstorben  
 bei Erkrankung  
 bei Unfall  
 bei sonstiger Todesursache

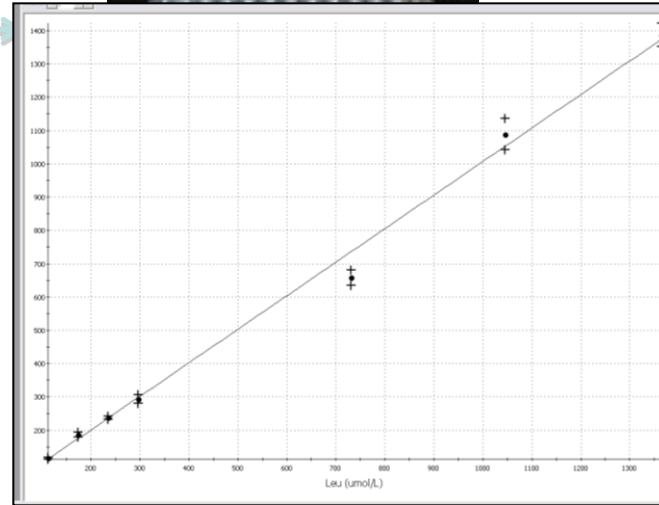
Screening-ID  
 Abstrakt  
 Privat

Daten des Kindes:  
 Baby's surname  
 Baby's Forename  
 Birth Date/Time  
 Spaceman Date/Time  
 Birth weight  
 Geschlecht  
 M  
 F  
 Gestaltage  
 Ist  
 Ist nicht  
 Ist nicht bekannt

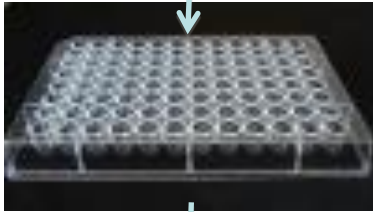
Physician's / Hospital Name, Address, Physician's Phone No.  
 Geburtsnummer der Mutter mit Vorwahl  
 Geburtsnummer des Kindes mit Vorwahl  
 Commenrs  
 Has baby  
 Blood  
 transfused  
 since therapy  
 family history ?

G00013350  
 G00013350  
 Bitte vollständig ausfüllen

Repunch



Punch

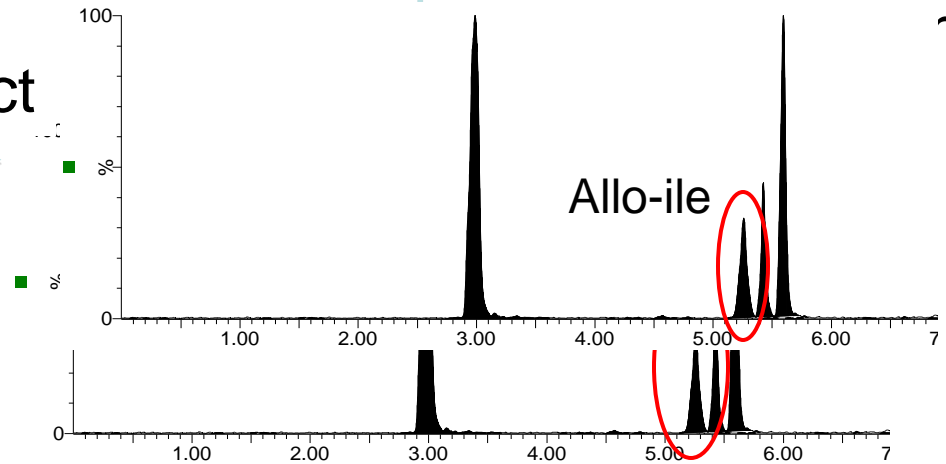
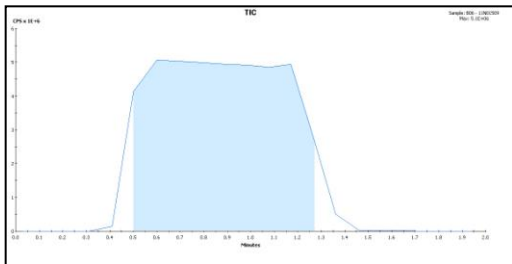


Extract  
Derivatize



Reinject

Inject

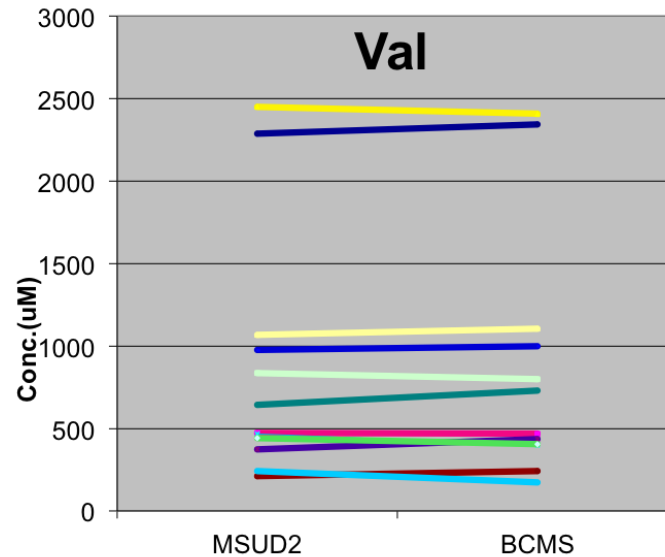
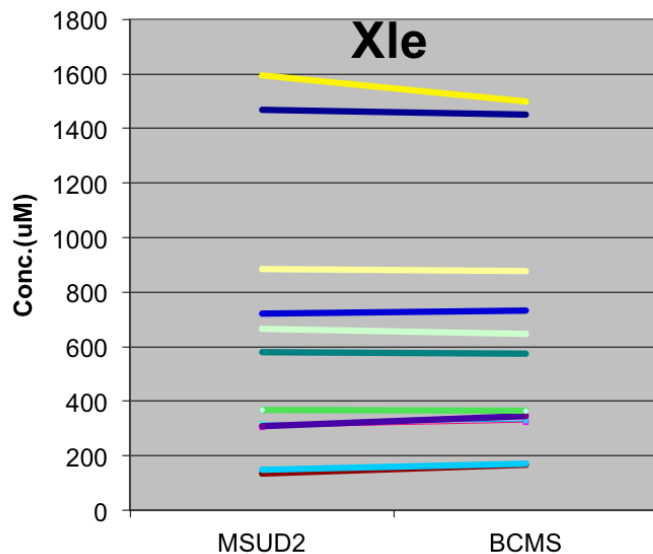


Calibators, QC, Positive samples

7)

# Test Performance

Precision (n=5)	Leu	Ile	Allo-ile	Val
Intra-assay (%CV)	5.4	7.2	5.0%	5.1%
Inter-assay (%CV)	8.4	6.5%	8.8%	5.8%
Limit of detection (uM)	0.75	0.5	1.0	20
Linearity ( $R^2 > 0.98$ )	0.5-2000 uM			

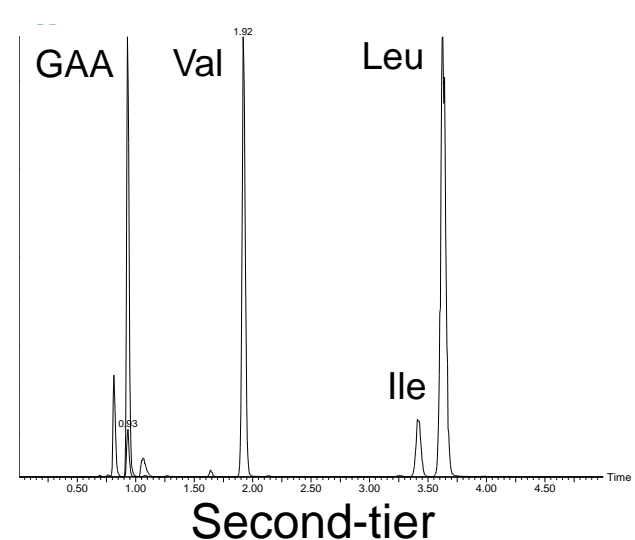
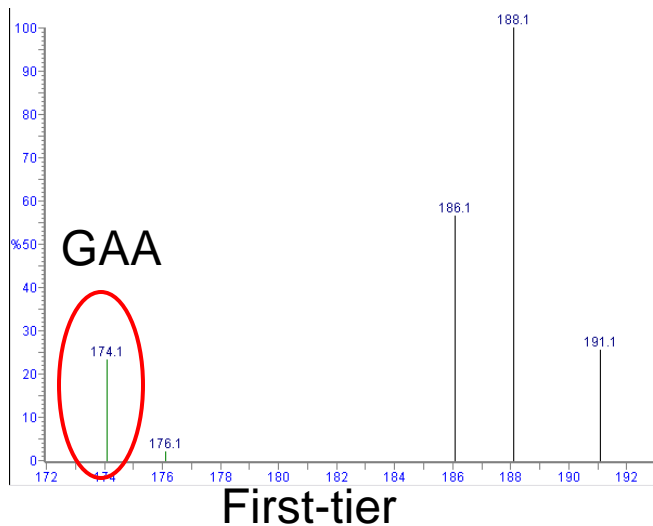


# Integrated MSUD Second-Tier

- Implemented Nov. 2010
- Lower first tier cutoffs (Xle and Val)
- Second Tier
  - Allo-ile  $>2$   $\mu\text{M}$  OR (Leu  $>200\mu\text{M}$  or Val  $>220\mu\text{M}$ )
- Increase theoretical sensitivity  $>95\%$
- In first year (N=44,822):
  - 60 samples to second tier, 3 positive cases
  - 2 false positive (no allo-ile, TPN confirmed)
  - 1 true positive (PPV 30%)

# Future Integrated Tests

- IVA? (Separate C5 isomers, pivalic acid)
- GAMT Screening:
  - First-tier GAA with current AA/AC method
  - False positives from isobaric compound (?)
  - Second-tier GAA with chromatography
    - Can be combined with MSUD second-tier



# Acknowledgements

- Hilary Vallance (Program Director)
- Daisy Baulcomb (Lab Supervisor)
- Judie Nelson (R&D Technologist)
- NBS Staff
- Region 4 MS/MS Collaborative project



a place of mind  
THE UNIVERSITY OF BRITISH COLUMBIA



Perinatal  
Services BC  
An agency of the Provincial  
Health Services Authority

