

Second tier tests and newborn screening

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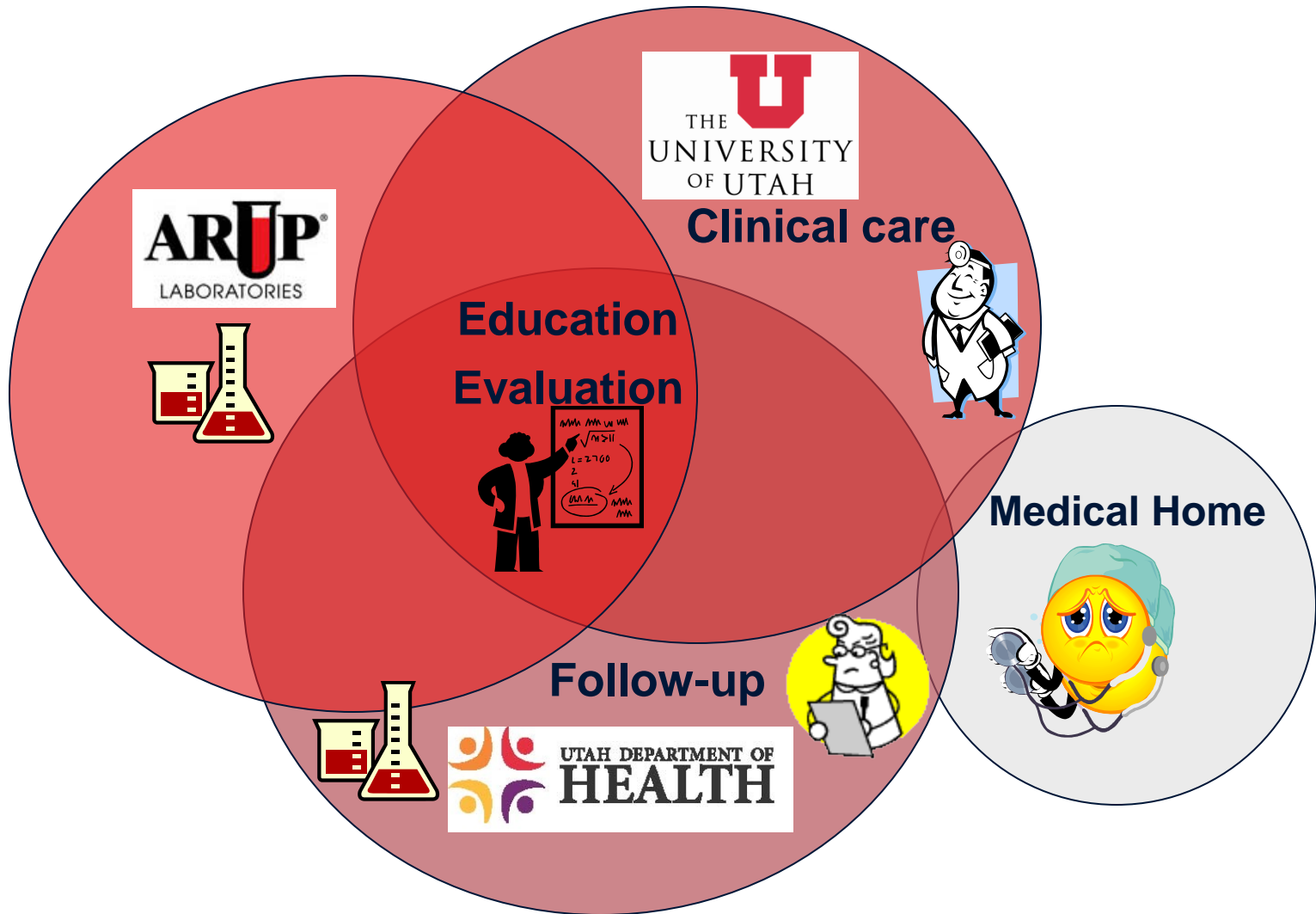
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Outline

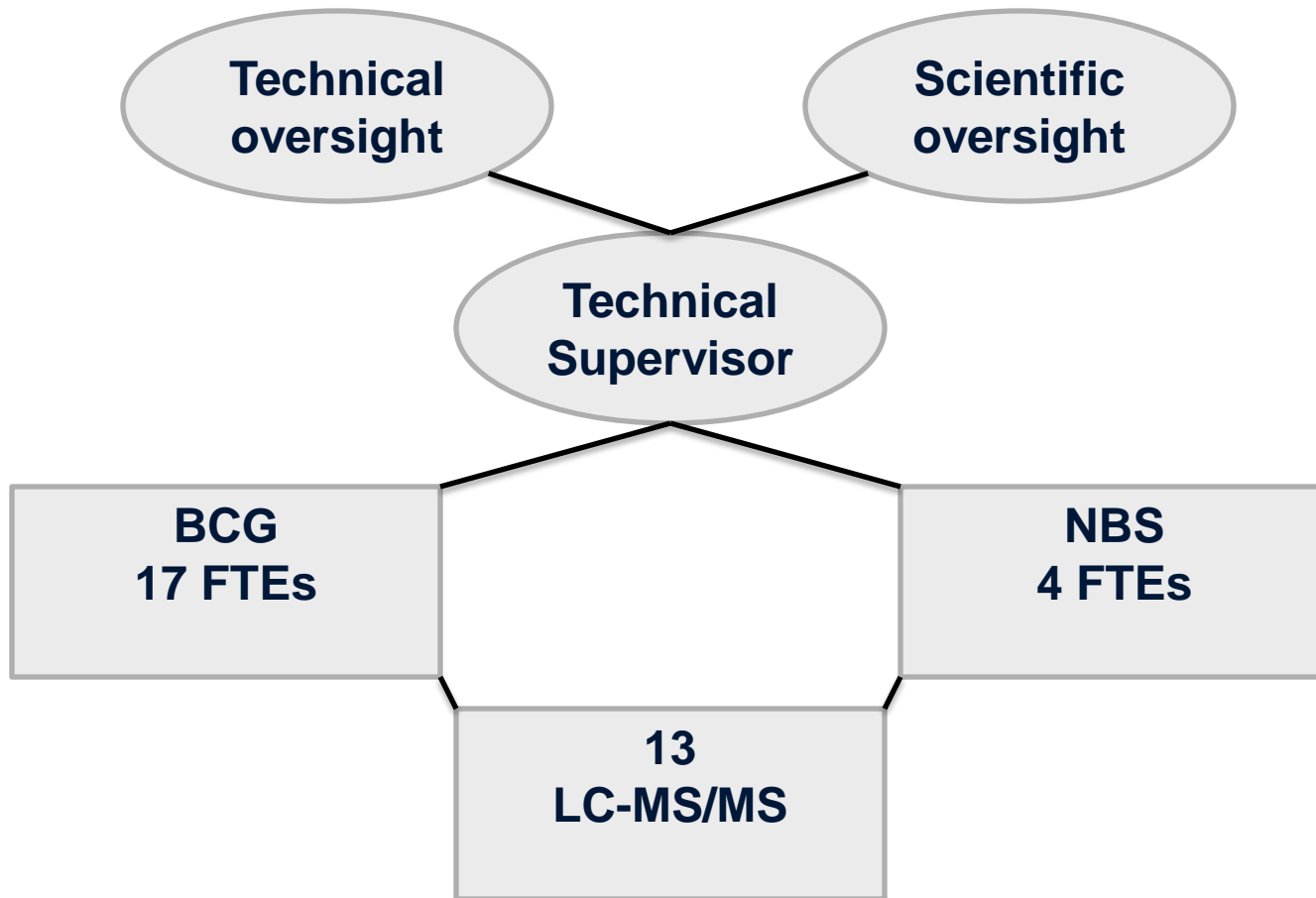
- **Utah Newborn Screening Program**
- **ARUP Newborn screening laboratory**
- **Second tier tests algorithms/workflow**

UTAH Newborn screening program



The efficiency and effectiveness of a newborn screening program is dependent upon the smooth integration of sample collection, laboratory testing, follow-up, diagnosis, timely treatment, and tracking of outcomes.

Biochemical Genetics and Newborn Screening Laboratories at ARUP



Second Tier Tests

- **Tests run on the SAME sample used for the primary screen**
- **Different target analytes**
- **Often a different methodology is used**

What is the purpose of second tier tests?

- **Identify infants at risk of having a metabolic condition, while**
- **Reducing false positives (proportion of non-affected individuals who test positive), and**
- **Reducing false negatives (proportion of true affected individuals who test negative)**

Impact of false positive results

- **Anxiety**
- **Increased costs to parents**
- **Increased costs to society**
- **Decreased credibility for NBS program**
- **“Cry wolf” effect**
- **Potential for missing appropriate follow-up of a real patient**

Impact of false negative results

- **Missing a diagnosis of a potentially treatable metabolic condition, resulting in**
- **Morbidity and mortality associated with the condition**

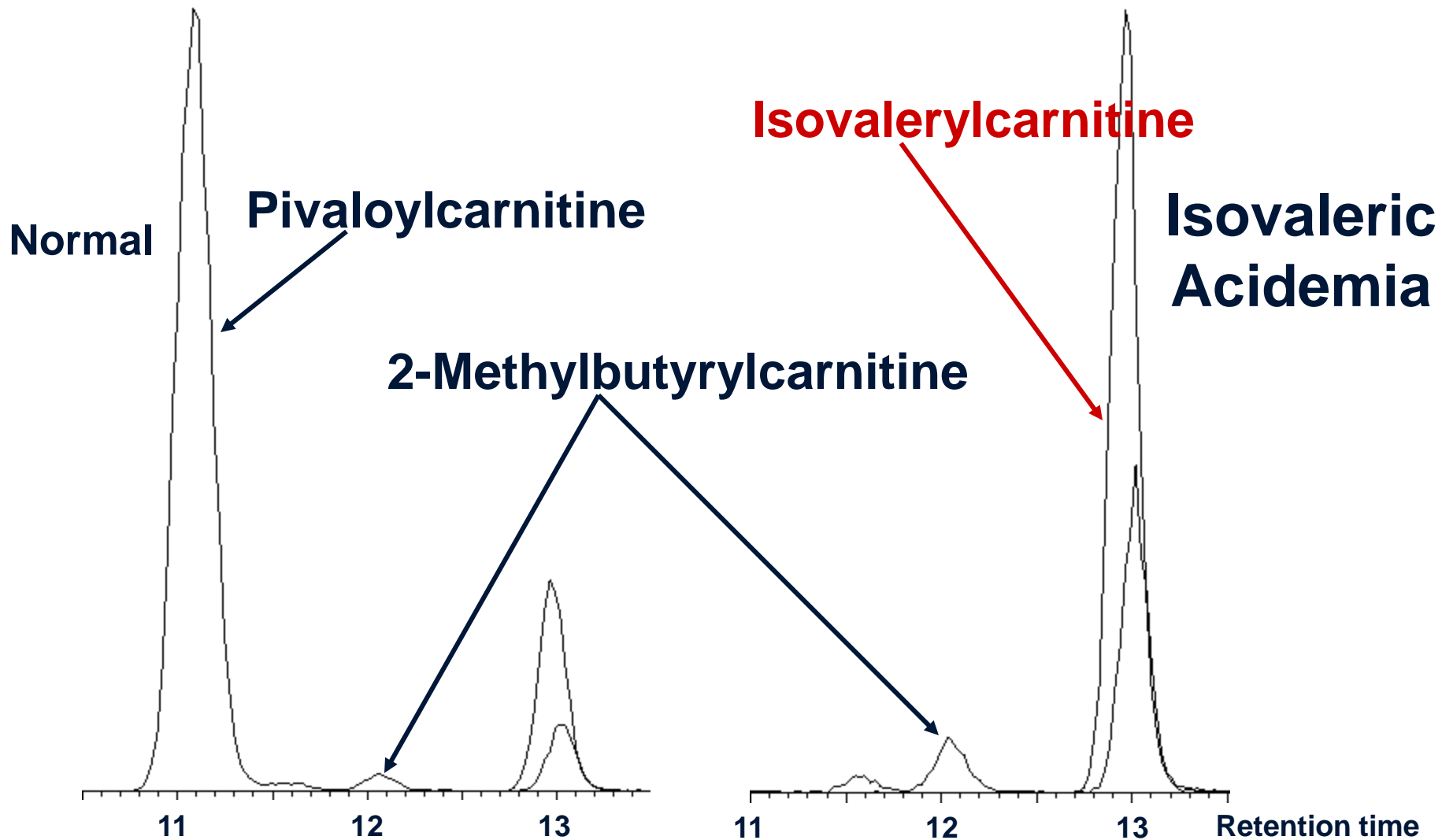
Second tier tests and newborn screening

- **Biochemical/small molecule analysis based tests**
 - **LC-MS/MS**
- **Molecular testing**
 - **Cystic Fibrosis**
 - **Lysosomal storage diseases**
 - **DNA testing for metabolic disorders**

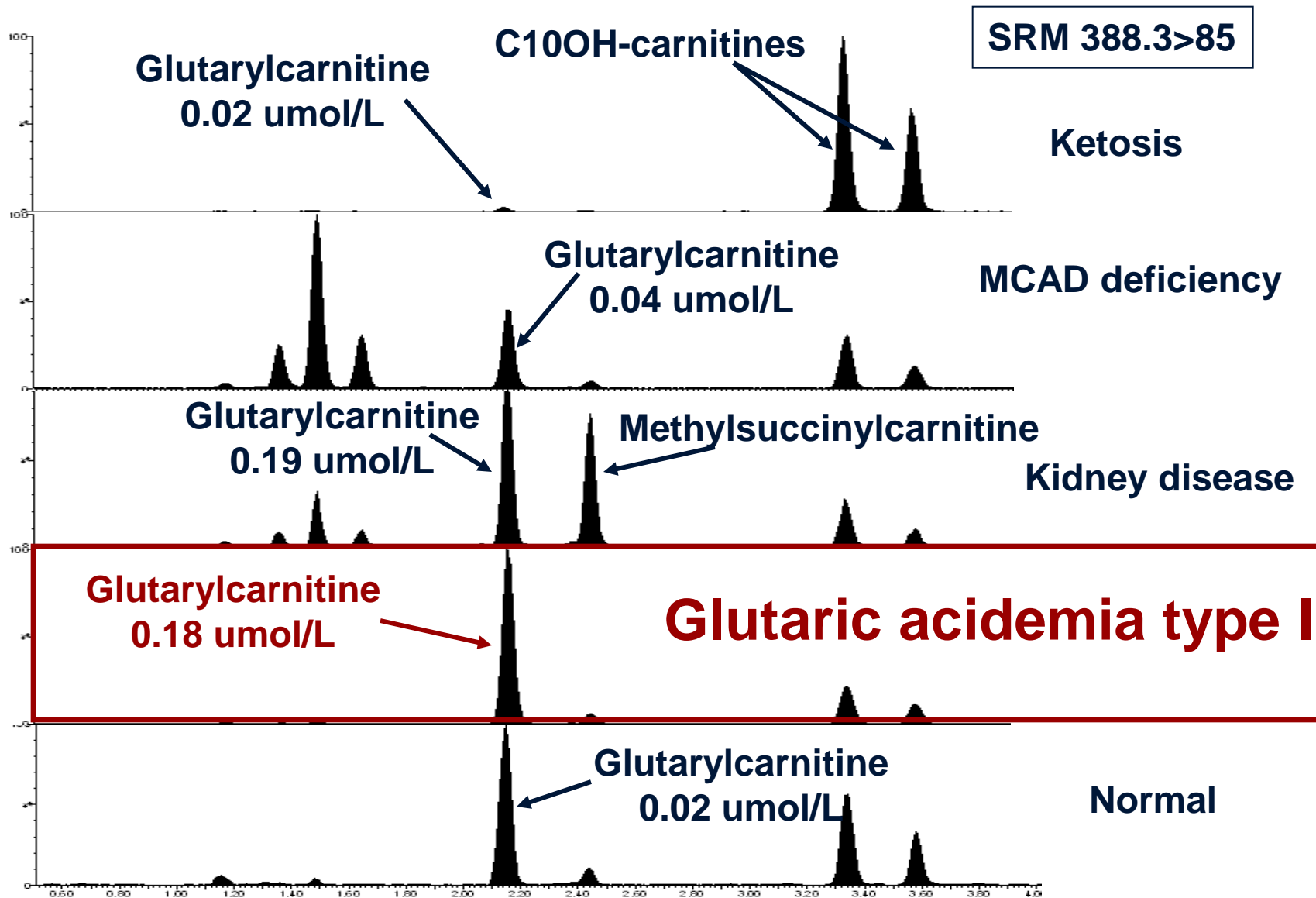
Strategy for 2nd tier tests

- **Presence of compounds that produce ions with the same mass/charge ratio: chromatographic separation**
 - **Antibiotics**
 - **Isomers/Isobars (allo-isoleucine, hydroxyproline, C10-OH-carnitine)**

2nd tier test for elevated C5-carnitine



2nd tier test for elevated C5DC-carnitine

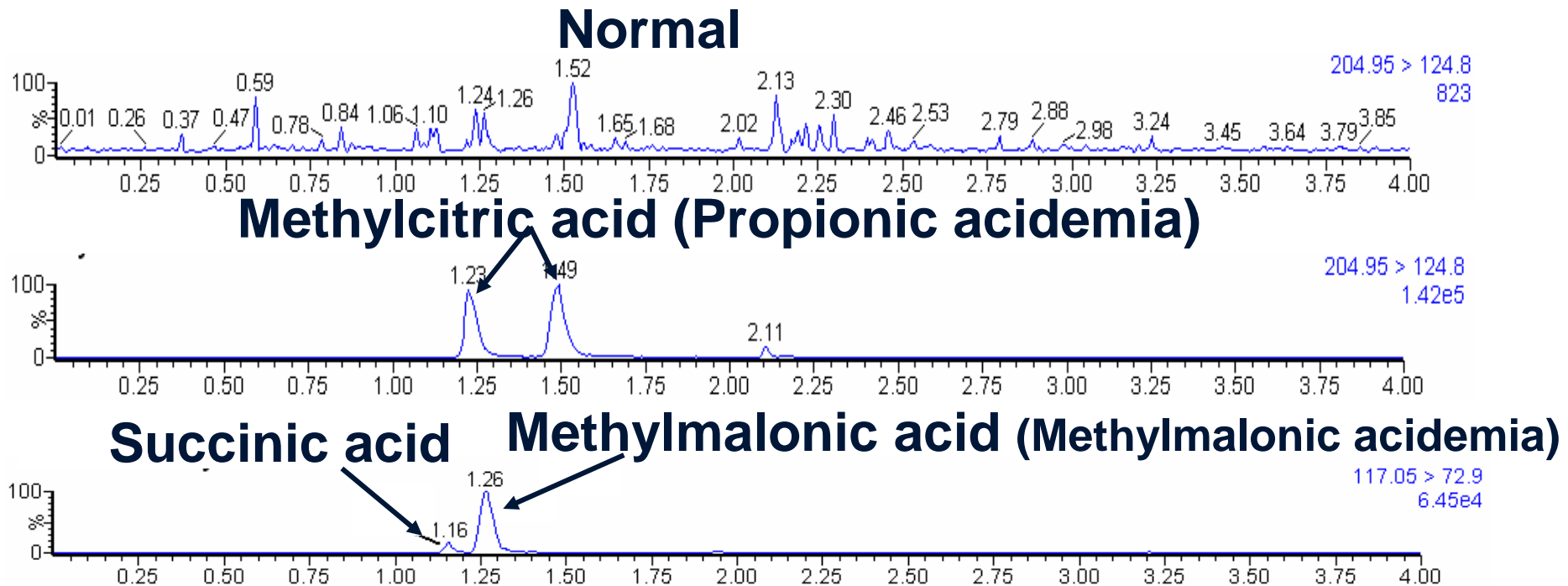


Strategy for 2nd tier tests

- **Specific markers for metabolic conditions:**
 - **Elevated C3-carnitine**
 - Methylmalonic, methylcitric, 3-hydroxypropionic acids
 - Total homocysteine
 - **Elevated methionine**
 - Total homocysteine
 - **Low methionine**
 - Methylmalonic acid, total homocysteine

2nd tier test for elevated C3-carnitine

- Specific markers for metabolic conditions:
 - Elevated C3-carnitine
 - Methylmalonic/methylcitric acid



2nd tier tests available

- **Steroid profile for CAH**
- **Total homocysteine (elevated/low methionine)**
- **Glutaryl carnitine (elevated C5DC-carnitine)**
- **Methylmalonic/methylcitric acid (elevated C3)**
- **Allo-iso leucine (elevated Xle)**
- **Ethylmalonic acid (elevated C4-carnitine)**
- **Guanidinoacetate for GAMT deficiency**

2nd tier tests

- **Steroid profile for CAH (~150/month)**
- **Methylmalonic/methylcitric acid (~100/month)**
- **Glutaryl carnitine (~50/month)**
- **Total homocysteine (~30/month)**
- **Allo-isoleucine (~1/month)**
- **Ethylmalonic acid (~5/month)**
- **Guanidinoacetate for GAMT deficiency (?)**

Choice of second-tier tests

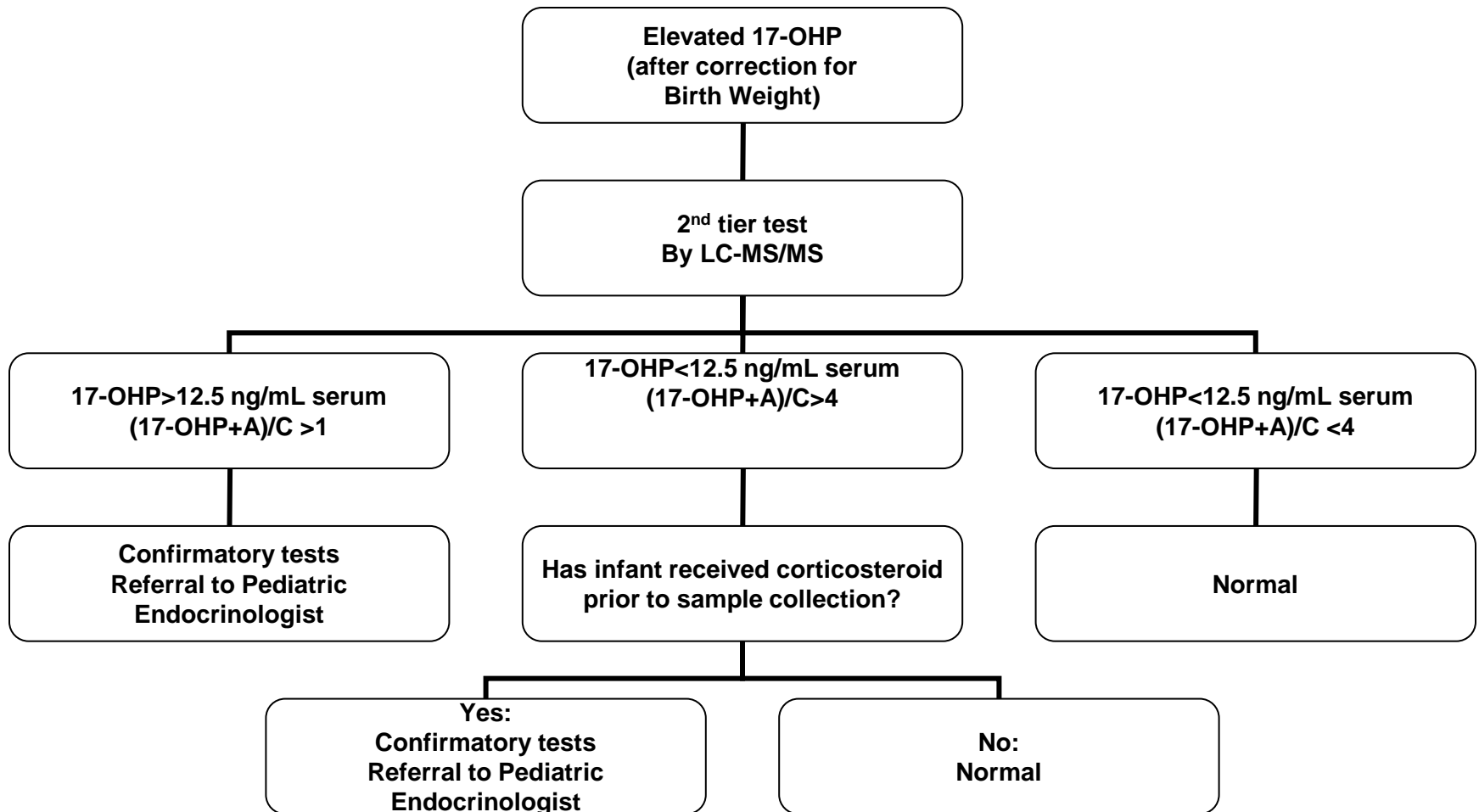
- **Identify the biggest “offender”**
 - **Positive predictive value for a specific marker**
(probability of being affected when the test is positive)

Year	Marker(s)	Condition	# of positives	# of diagnosis	PPV %
2006	Phe	PKU	11	10	91
	C3	PA, MMA	202	1	0.5

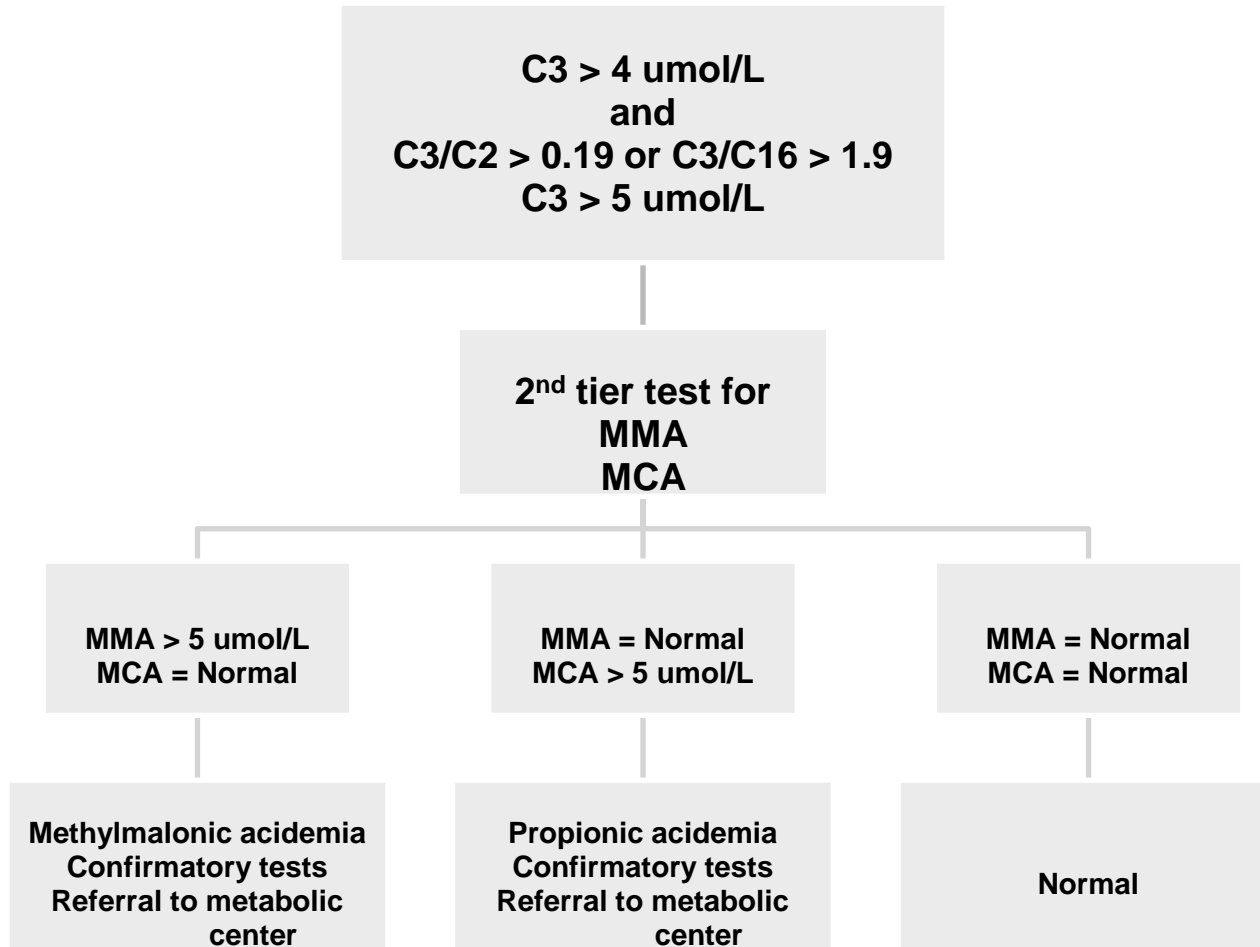
Effectiveness of second-tier tests

Year	Marker(s)	Condition	# of positives	# of diagnosis	PPV %
2013	Phe	PKU	7	6	86
	C3	PA, MMA	11	11	100

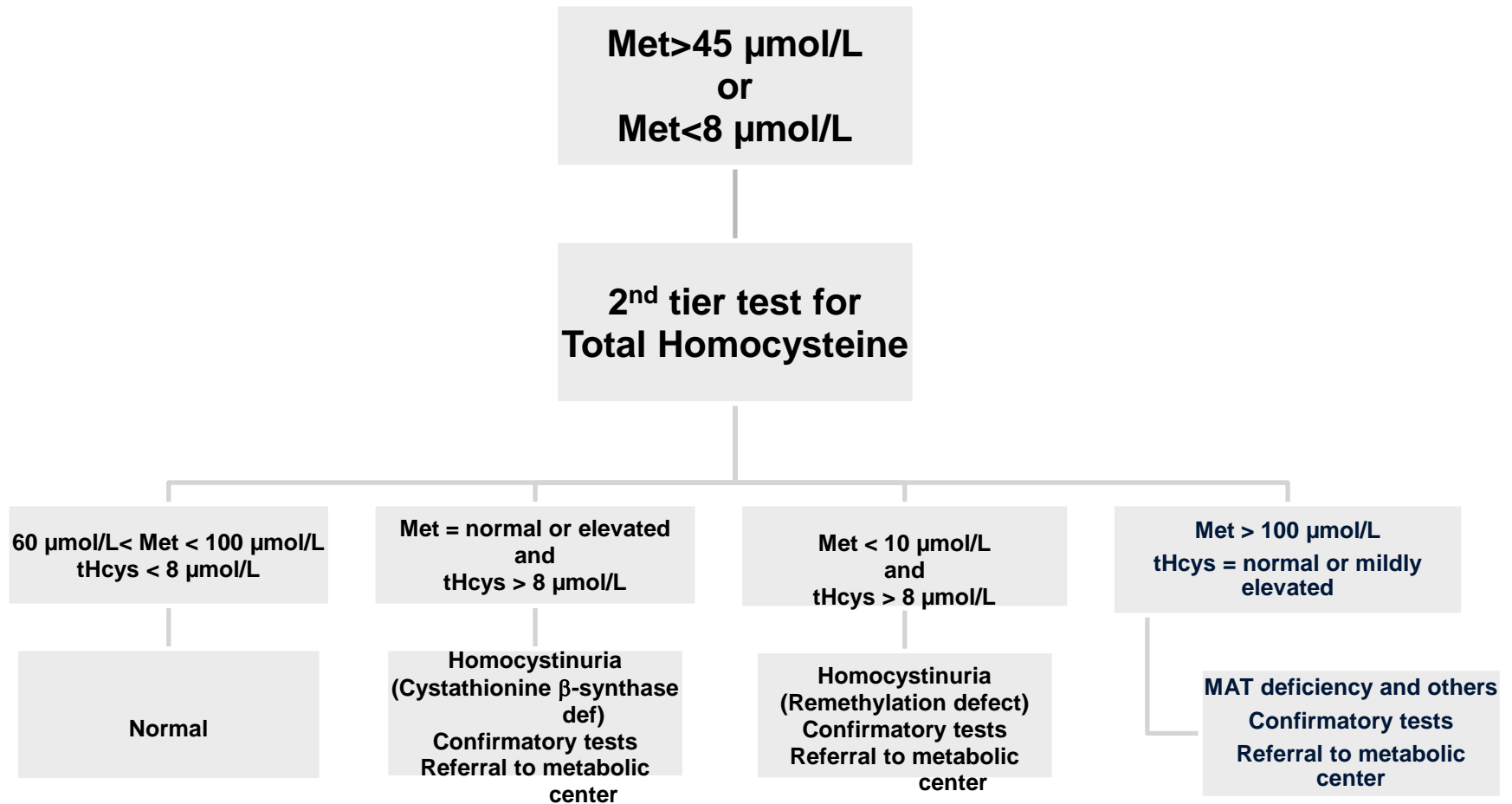
Algorithm for CAH



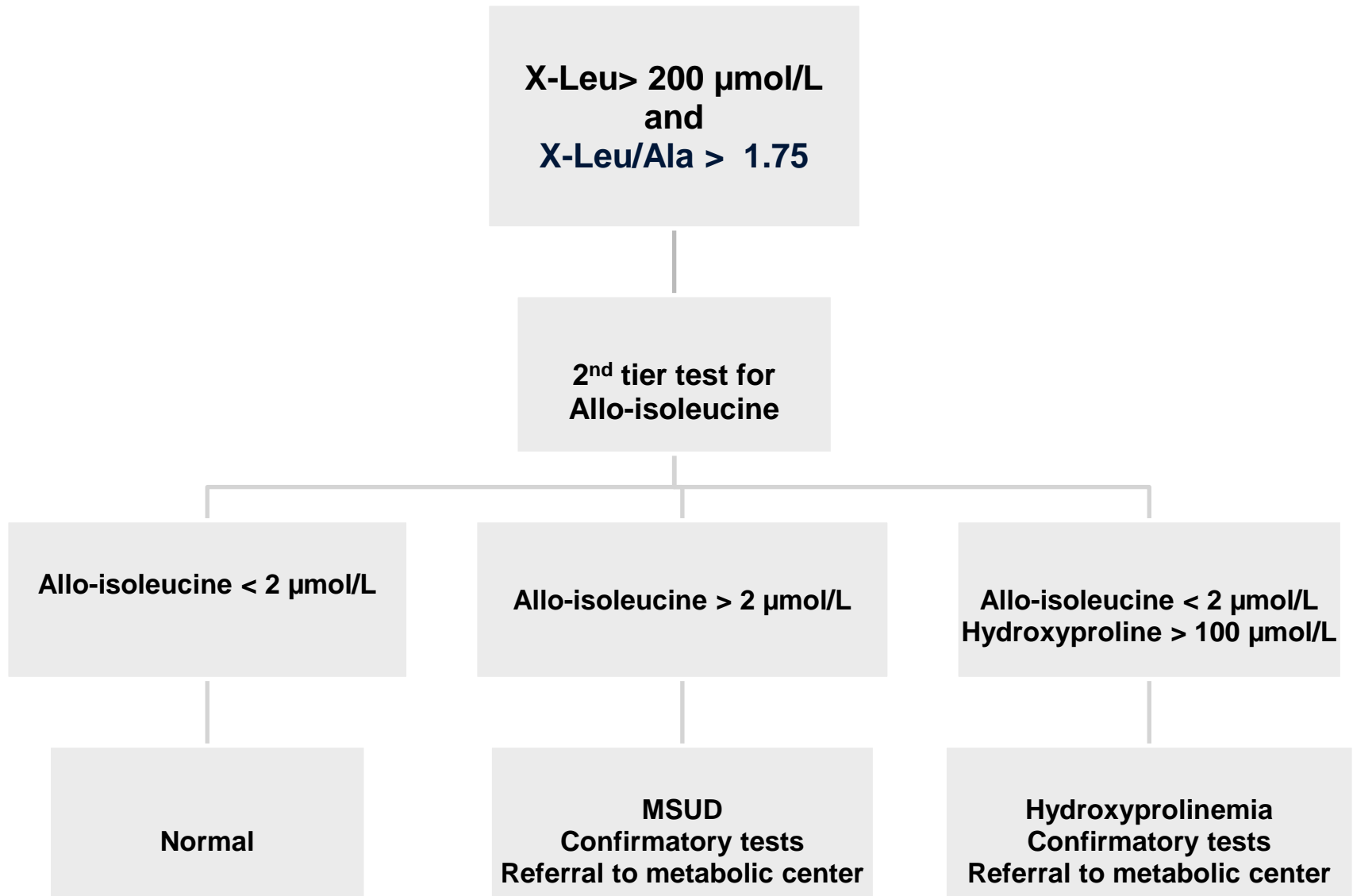
Algorithm for C3-carnitine



Algorithm for Methionine



Algorithm for X-Leu



Newborn screening workflow

- **Primary screen run and reported daily**
- **2nd tier tests:**
 - **CAH run daily**
 - **MMA/MCA run 3+ /week**
 - **Hcys run weekly**
 - **Allo-isoleucine run as needed**
 - **C5DC run as needed**

Barriers to implementation of 2nd tier tests

- **Availability of resources**
 - **Instruments**
 - **Personnel**

- **Cost effectiveness**
 - **Economy of scale**

Regional approach to 2nd tier testing

- **Evaluation and implementation of second tier testing for disorders identified by MS/MS in newborn blood spots in the Mountain States Region (CDC- grant 5U01EH000453-02)**
 - **Coordinate with the Mountain States (Region 6) the submission of samples (blood spots) to be analyzed with a second tier method.**
 - **Compare the number of positive results after the 2nd tier tests with the number of positive results obtained with the primary screen.**
 - **Evaluate the feasibility of a regional center for second tier tests.**

Region 6: 2nd tier tests

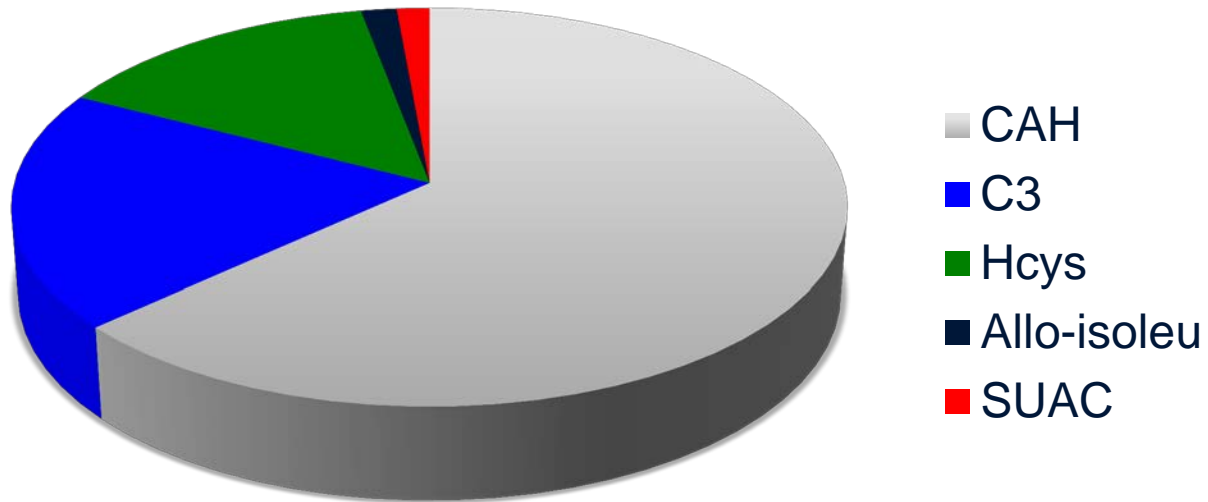
- **Steroid profile for CAH**
- **Methylmalonic/methylcitric acids for elevated C3-carnitine**
- **Total homocysteine for high methionine**
- **Allo-isoleucine for MSUD**
- **Succinylacetone for Tyrosinemia type I**

Utah: 2nd tier tests

- **Steroid profile for CAH**
- **Methylmalonic/methylcitric acids for elevated C3-carnitine**
- **Total homocysteine for high methionine**
- **Allo-iso-leucine for MSUD**
- **C5DC for Glutaric acidemia type I**
- **Ethylmalonic acid for SCAD**

Region 6 study: results

- **Results: 9650 second tier tests**



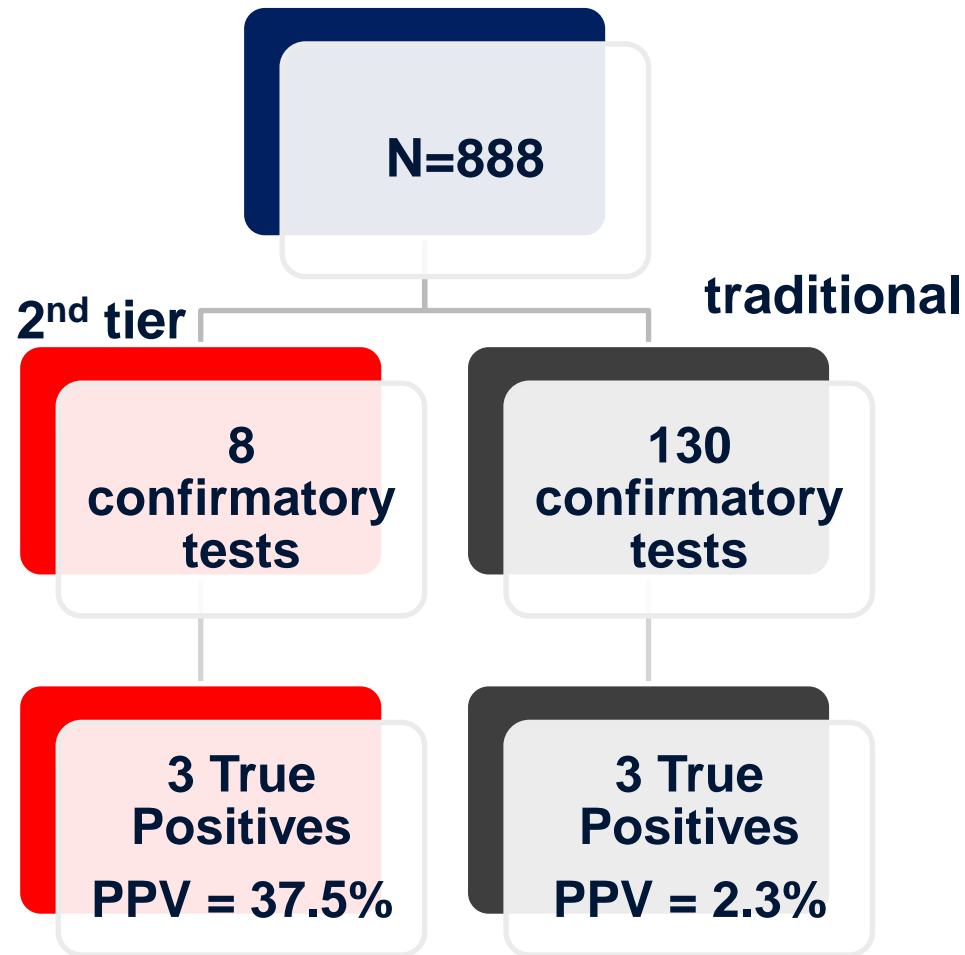
Region 6 study: results

- Proportion of tests run on babies weighing <2,000 g at birth:
 - Allo-isoleucine (Xle) 38%
 - Total homocysteine (Met) 37%
 - Succinylacetone (Tyr) 33%
 - CAH 17%
 - Methylmalonic/methylcitric 14%
 - Ethylmalonic 2%

Region 6 study: results

- **Abnormal 2nd tier results by test**
 - **HCY** **1 (0.1%)**
 - **MMA/MCA** **8 (1%)**
 - **CAH** **91 (3.1%)**
 - **SUAC** **0**
 - **Allo-isoleucine** **0**
 - **EMA** **0**

2nd tier for methylmalonic/methylcitric acid



2nd tier test for CAH (Utah)

- **4 years data**
 - **Number of infants screened = 271,784**
 - **Number of abnormal 2nd tier tests requiring confirmatory tests = 58**
 - **Number of true positives = 23**
 - **False positive rate = 0.013%**
 - **Positive Predictive Value = 39.7%**

Summary

- **Second tier tests are effective in reducing false positives.**
- **Implementation of second tier tests can also reduce the stress to the NBS program, families, medical homes caused by false positives.**
- **They can be integrated in the laboratory workflow provided adequate instrumentation and personnel resources are available.**

Acknowledgments

- **MSGRC (Region 6)**
- **Utah Department of Health**
- **BCG and NBS laboratory at ARUP**
- **University of Utah Metabolic Center**