Newborn Screening for the Homocystinurias
(Classical Homocystinuria and Remethylation Disorders)
Expanding and Improving Biomarkers and Algorithms

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MEDICAL SCHOOL

New England
Newborn Screening
Program

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Objectives

- Review our current biomarkers and algorithms for HCU Disorders
-  Describe development and optimization of 2nd tier LC-MS/MS assay
- Discuss limitations and future considerations

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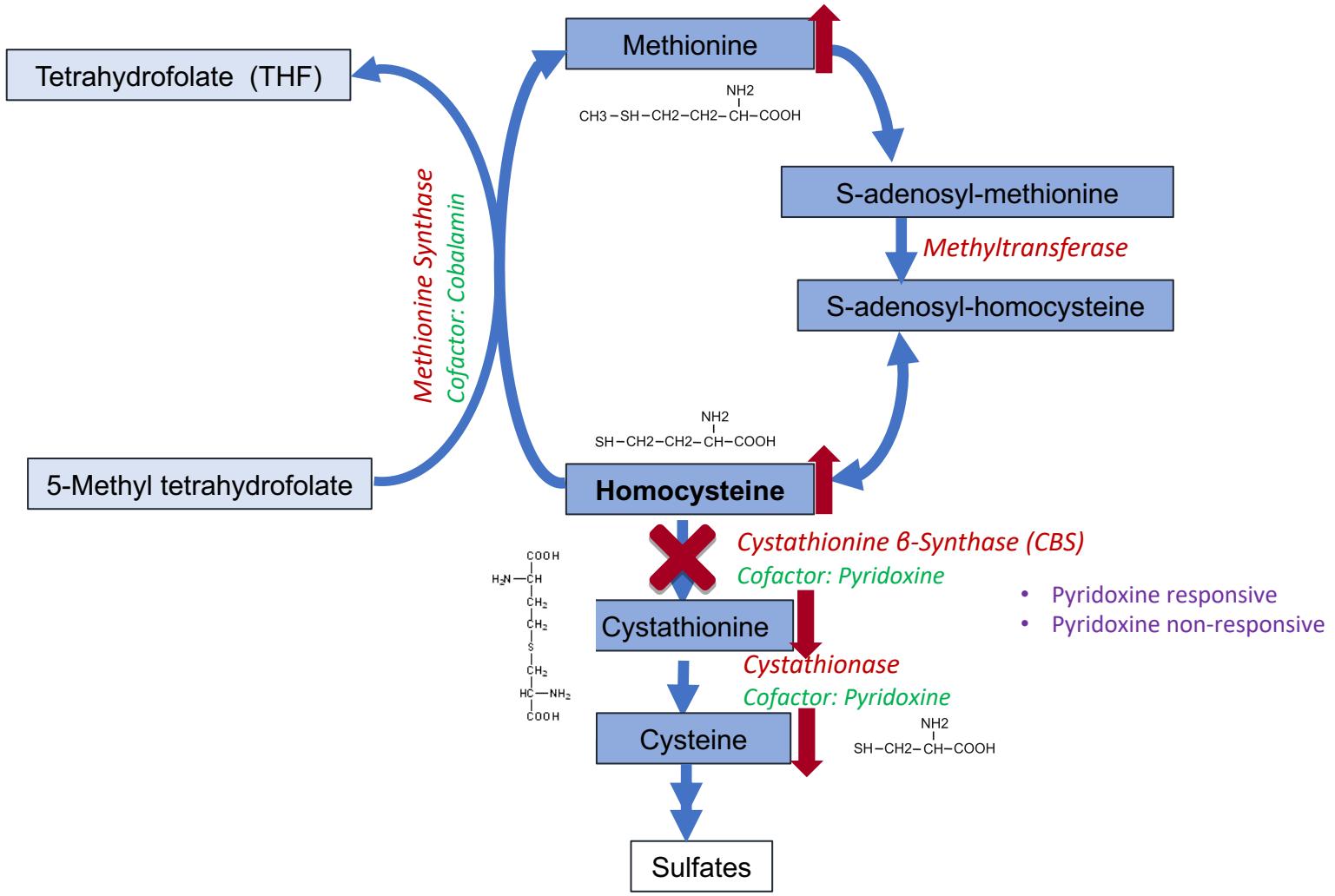
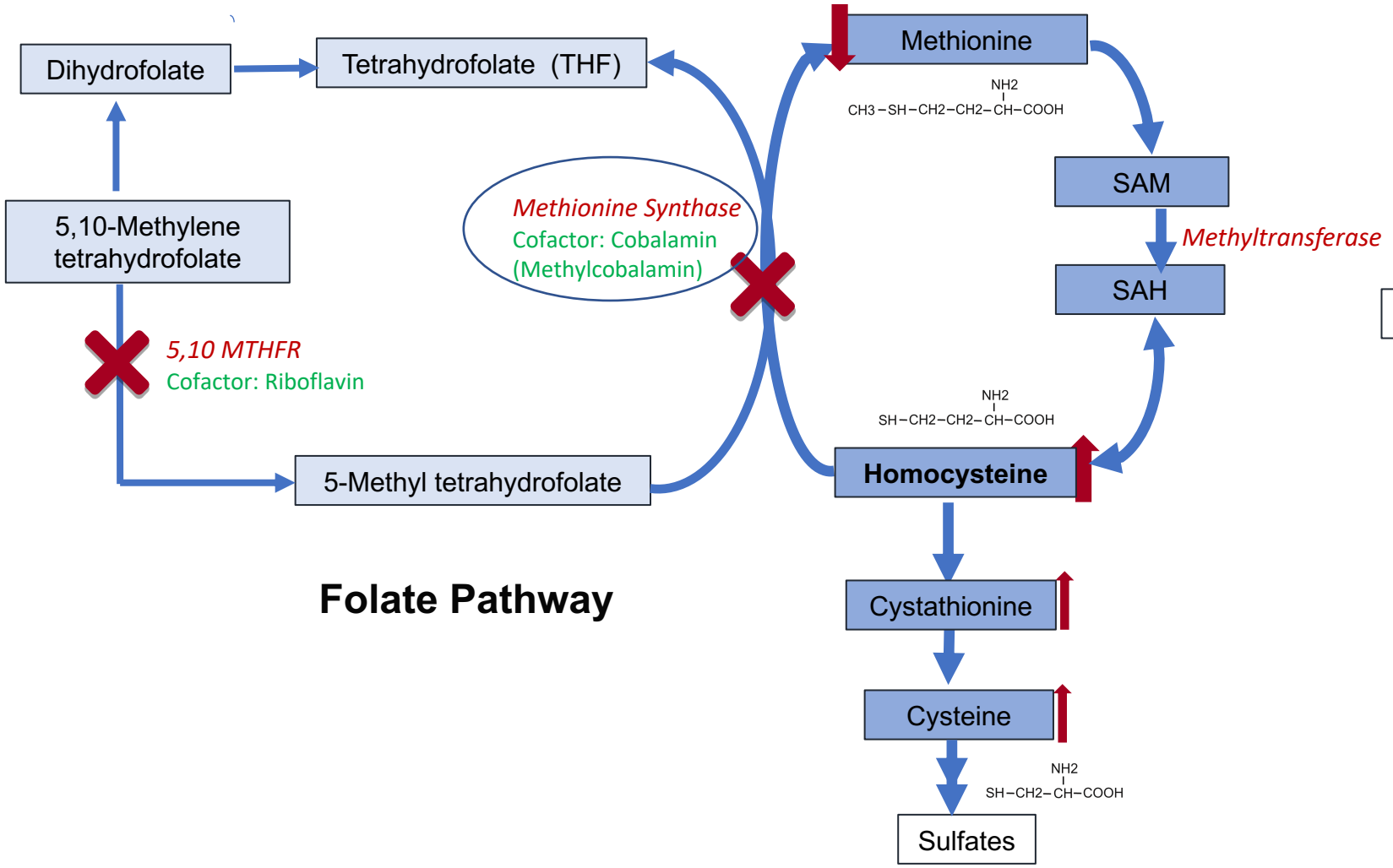
HCU Disorders – Classical HCU

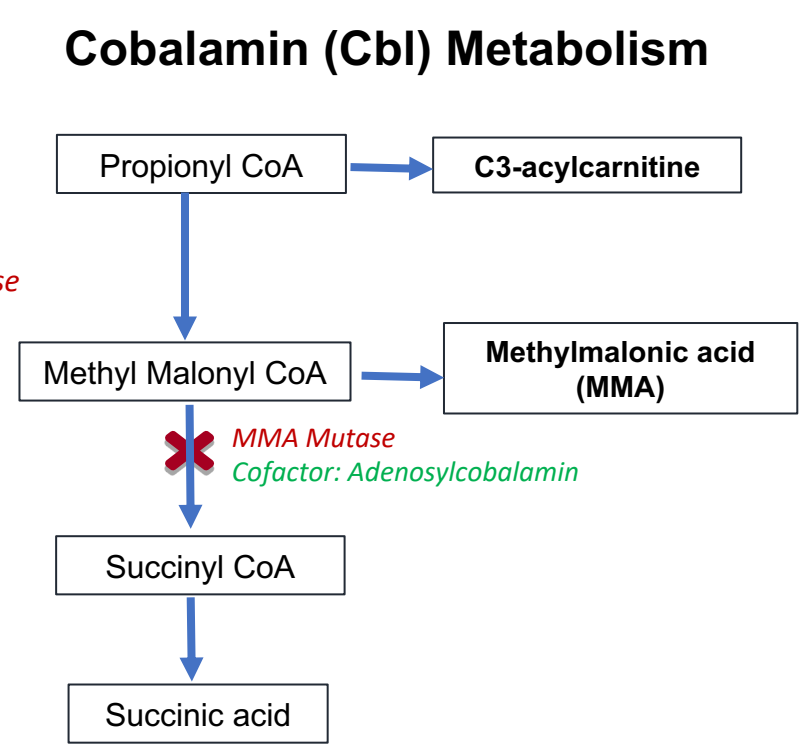
Defects in Trans-sulfuration Pathway



HCU Disorders – Remethylation Disorders

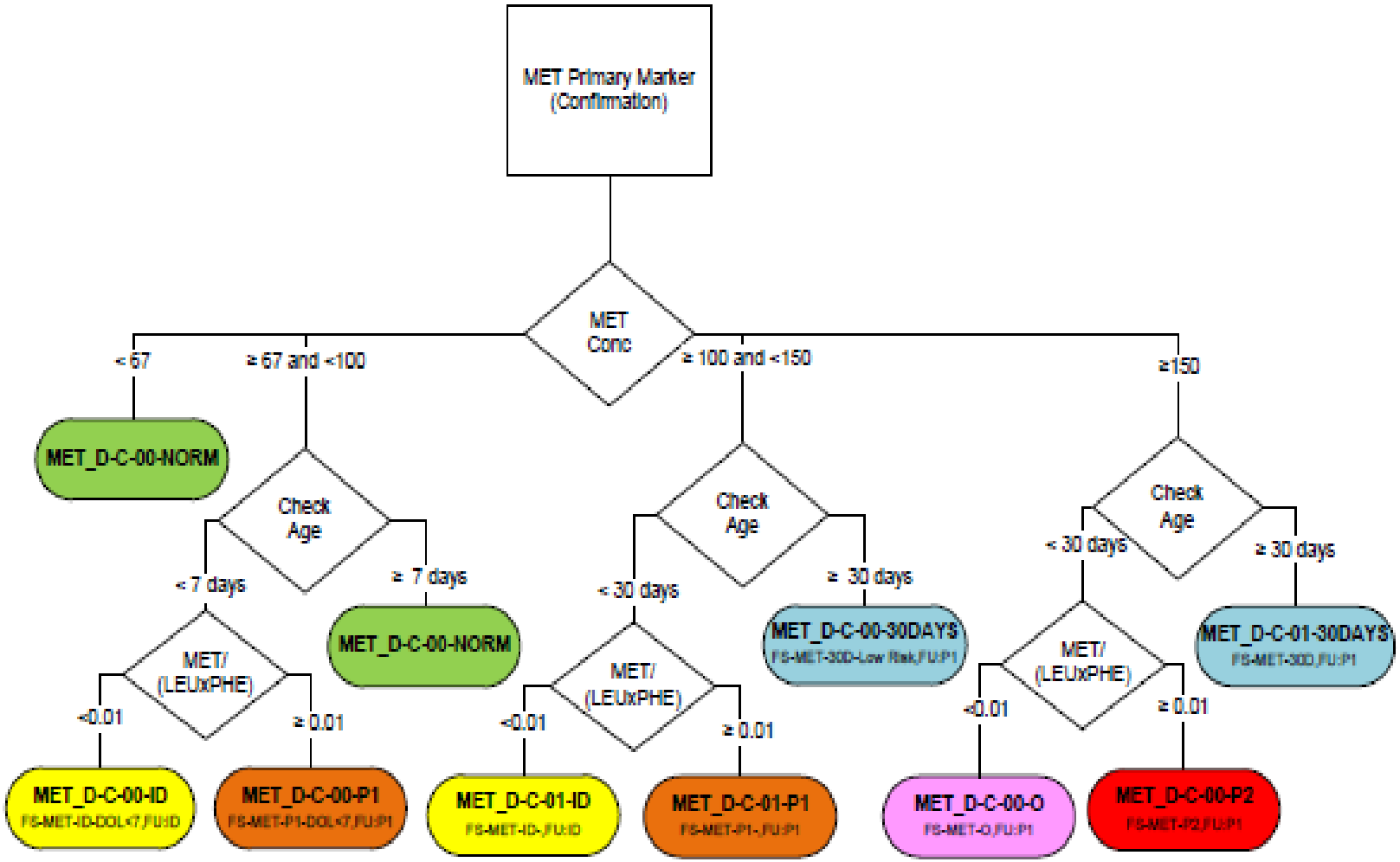
Defects in Remethylation Pathway





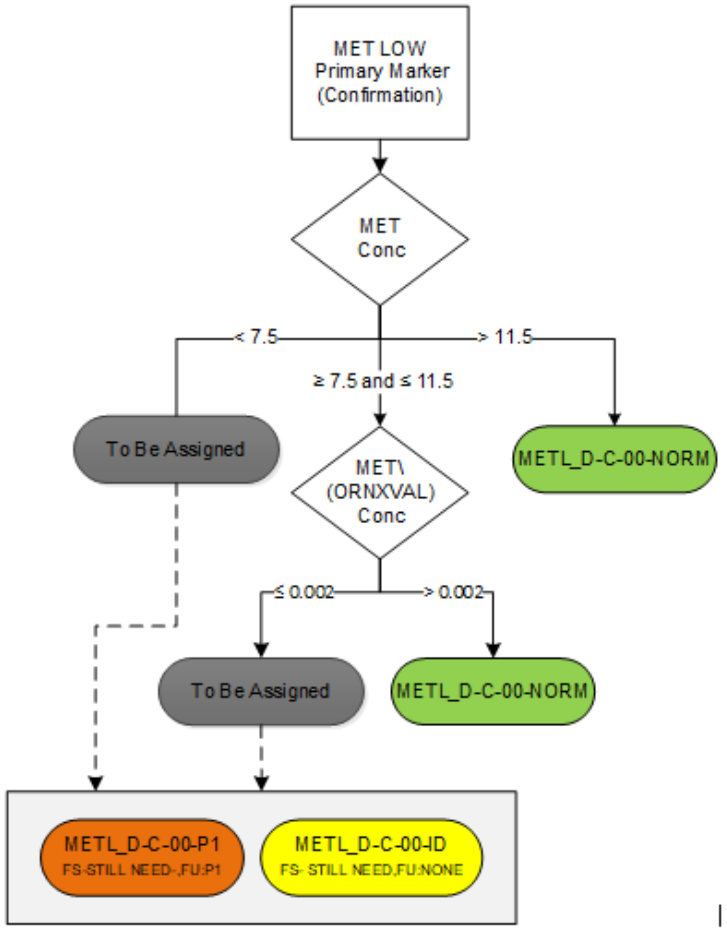
Current Screening and Algorithm for Classic HCU

Primary Marker: ↑Methionine

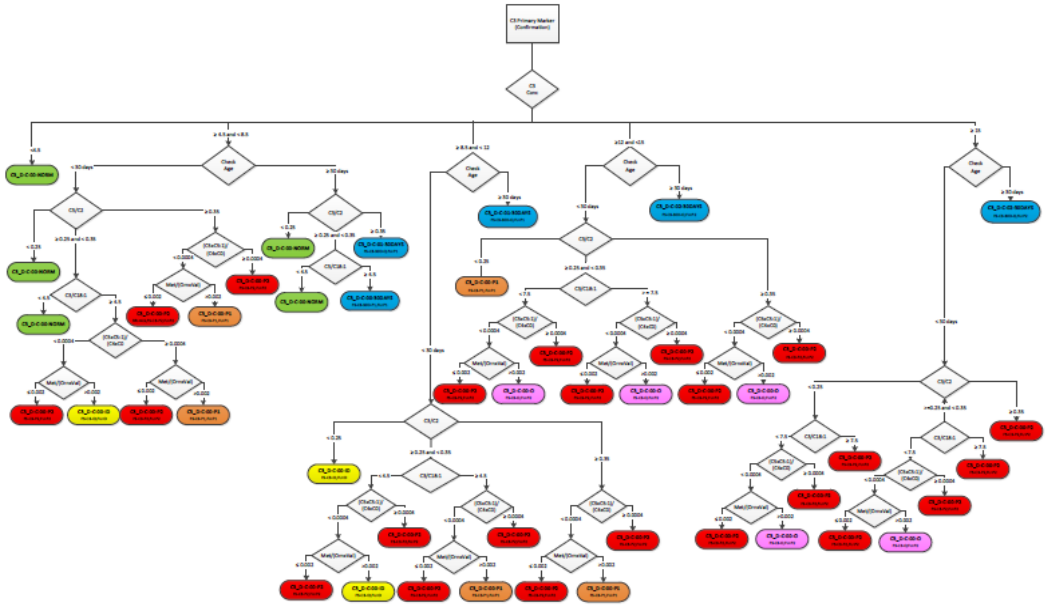


Current Screening and Algorithm for Remethylation Disorders

Primary Marker - ↓Methionine



↑C3



Strategies to Improve Screening Performance Classical HCU

Analytes	Specificity	Sensitivity
1. Decrease the ↑Met cut-off	↓	↑
2. #1 & Met/(Leu x Phe)	↑	↑
3. #2 & **2nd Tier** Hcy	↑↑	↑
4. #2 & **2nd Tier** Hcy, cystathionine & cysteine	↑↑↑	↑

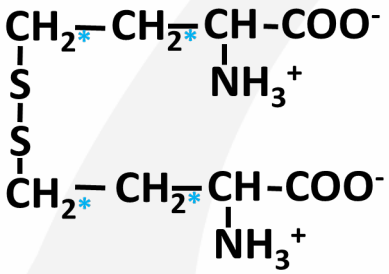
Strategies to Improve Screening Performance Remethylation Disorders

Analytes	Specificity	Sensitivity
1. Increase the ↓Met cut-off	↓	↑
2. #1 & Met/(Val x Orn)	↑	↑
3. #2 & **2nd Tier** Hcy	↑↑	↑
4. #2 & **2nd Tier** Hcy, cystathionine & cysteine	↑↑	↑
5. C3 & C3/C2* & 2nd Tier MMA	↔	↔

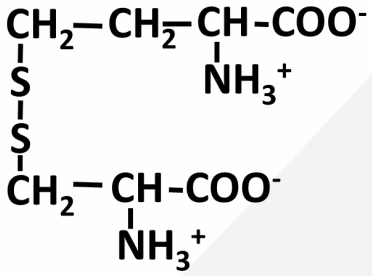
*Helps to distinguish CblC, CblD and CblF from other Remet disorders

Total Homocysteine (tHcy) and Total Cysteine (tCys)

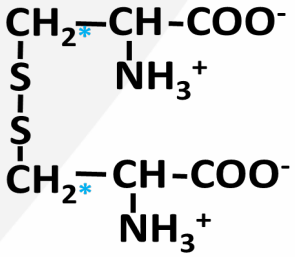
Homocystine



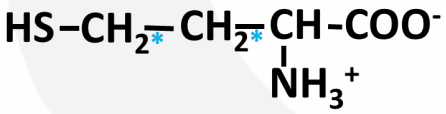
Homocysteine Cysteine disulphide



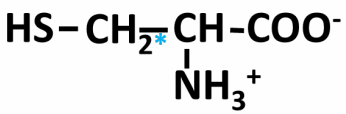
Cystine



Homocysteine



Cysteine

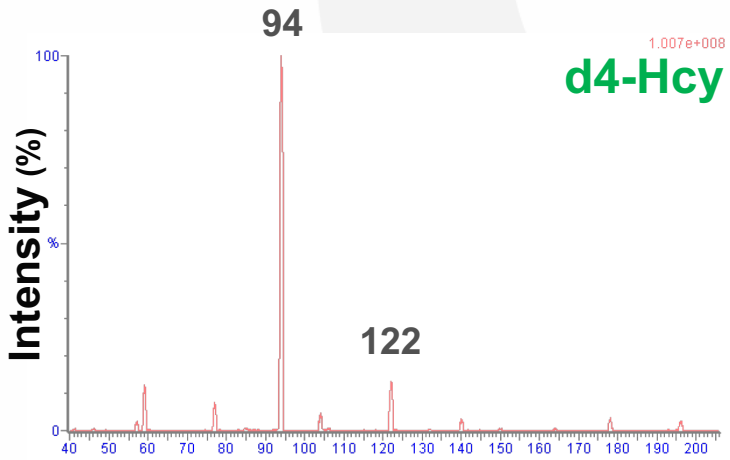


Specific Aims

- Develop, optimize, and validate a simple, robust and high throughput LC-MS/MS 2nd tier test: total homocysteine (tHcy), cystathionine, total cysteine (tCys) and MMA
- Determine reference ranges in neonates using DBSs
- Retrospectively analyze confirmed Classical and ReMet HCU disorders
- Assess, improve, and expand current algorithms for HCU using additional markers

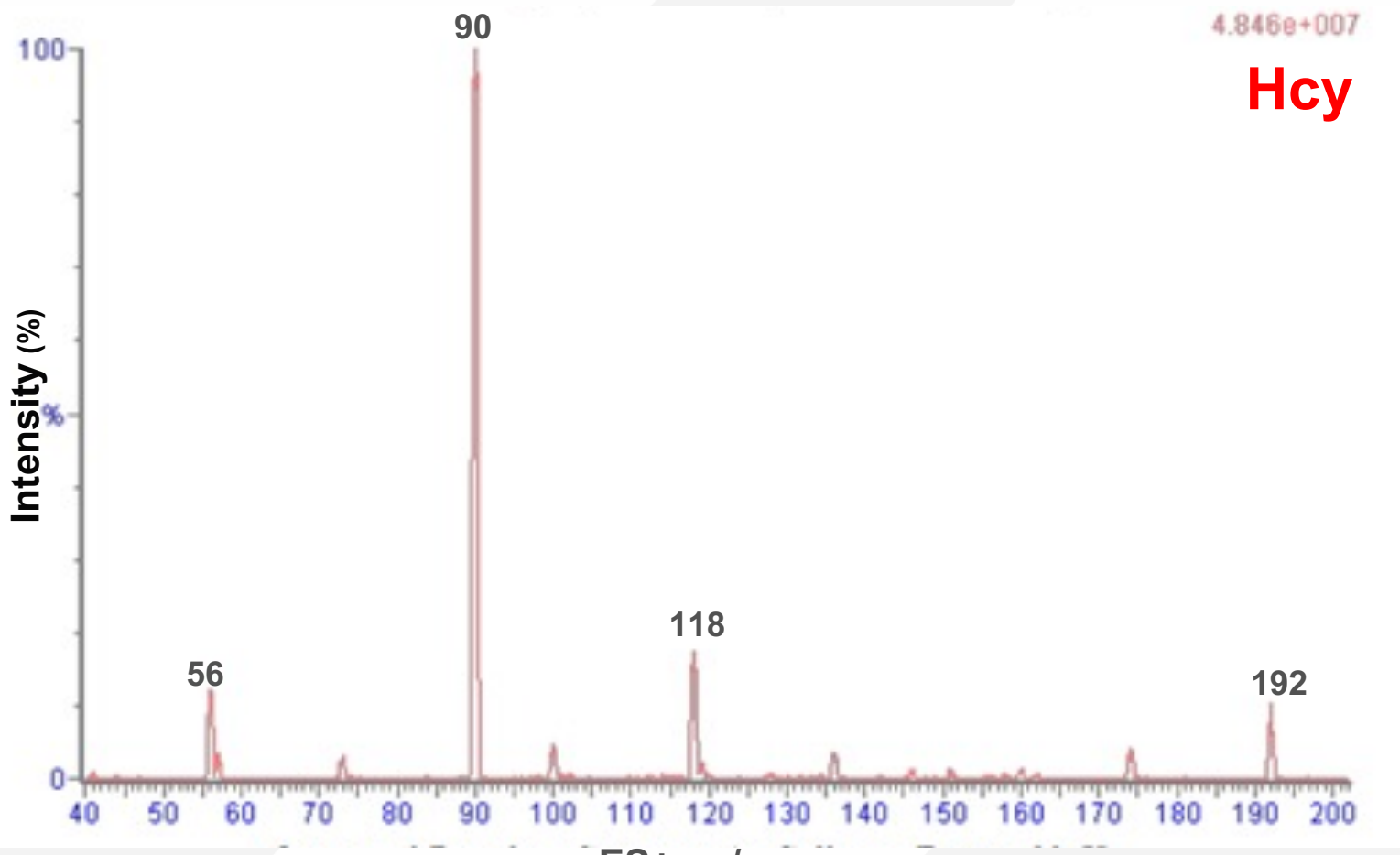
Optimized MS/MS Settings for Each Analyte and Its Deuterated Internal Standard on LC-MS/MS System

Analyte	Parent	Daughter	Cone (V)	Collision energy
Cysteine	178.0	104.9	20	9
d2-Cysteine	180.1	106.9	20	9
Homocysteine	192.0	90.0	25	12
d4-Homocysteine	196.0	94.0	25	11
MMA	231.1	119.0	18	9
d3-MMA	234.1	122.0	18	9
Cysta	335.2	190.2	24	15
d4-Cysta	339.3	194.1	12	13



+, m/z

Optimized Daughter Spectrum of Hcy

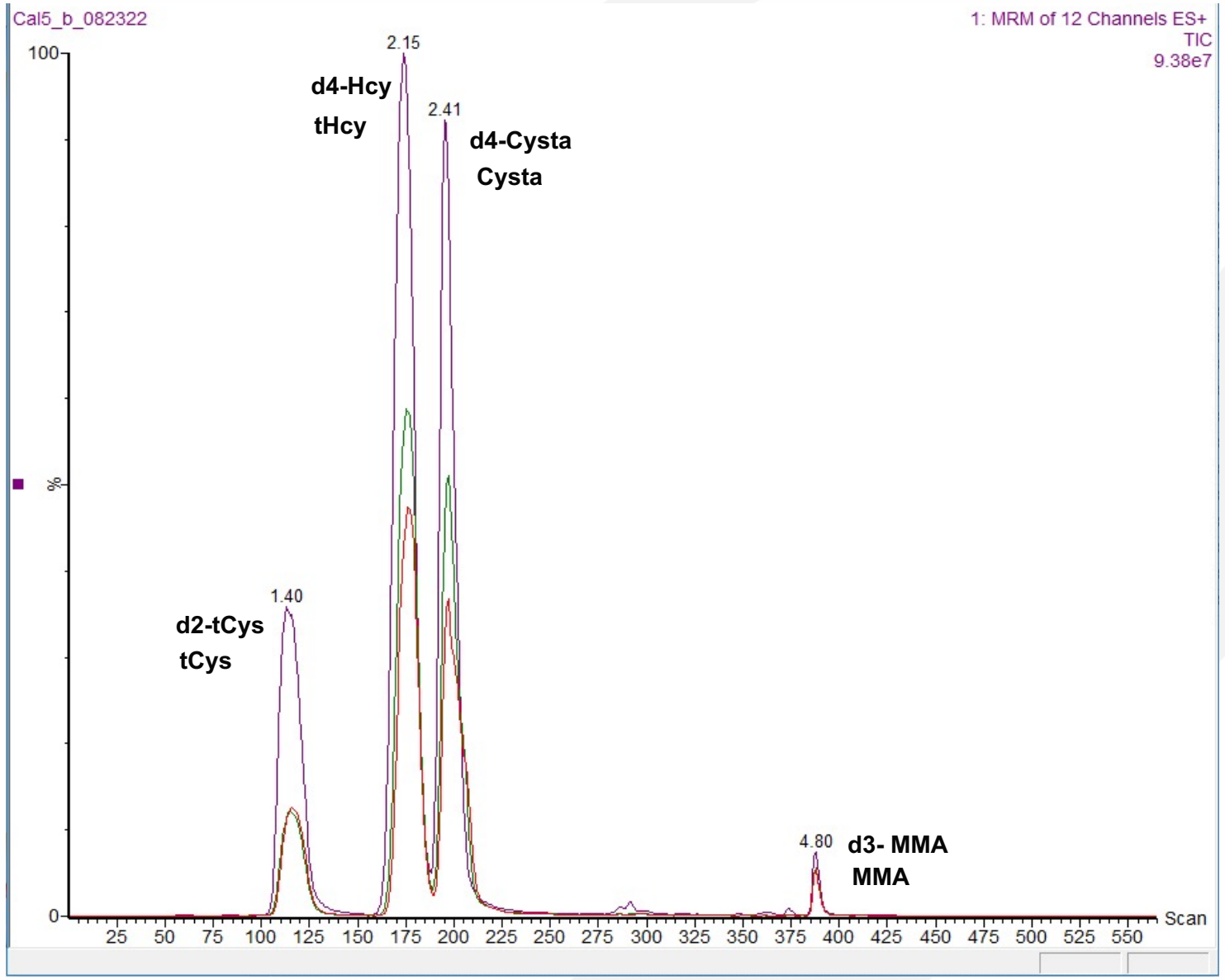


Optimizing Conditions for DBS Extraction

Derivatized method: Butyl Esters

- ✓**Solvents:** Methanol based, and Acetonitrile based
- ✓**Time:** Different extraction Time
- ✓**Temperature:** RT and 60ºC
- ✓**Reducing agent:** Different concentration
- ✓**Reconstitution Solvent:** Methanol based, and Acetonitrile based

Chromatogram Showing Separation of Analytes

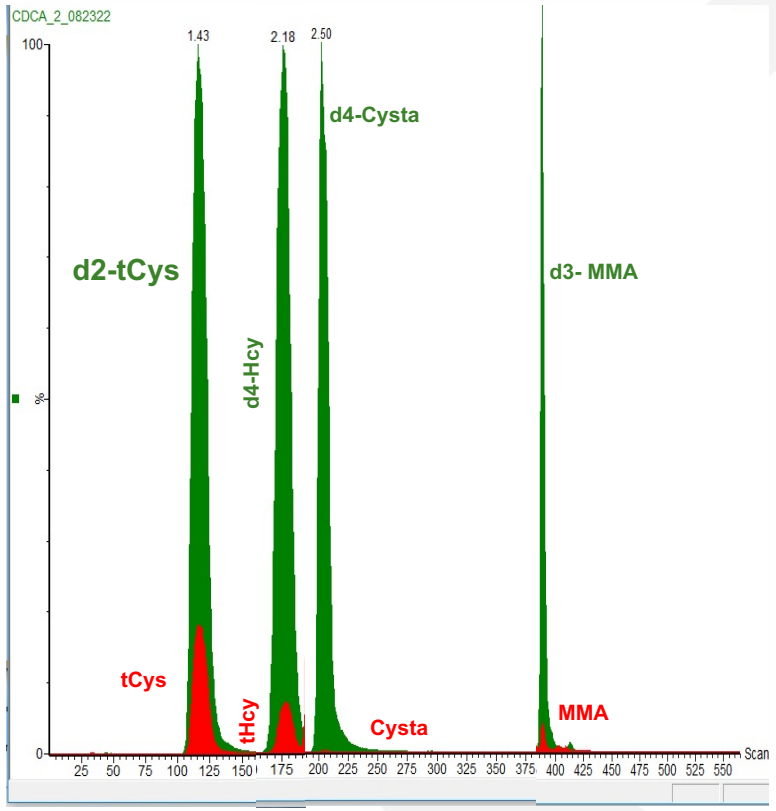


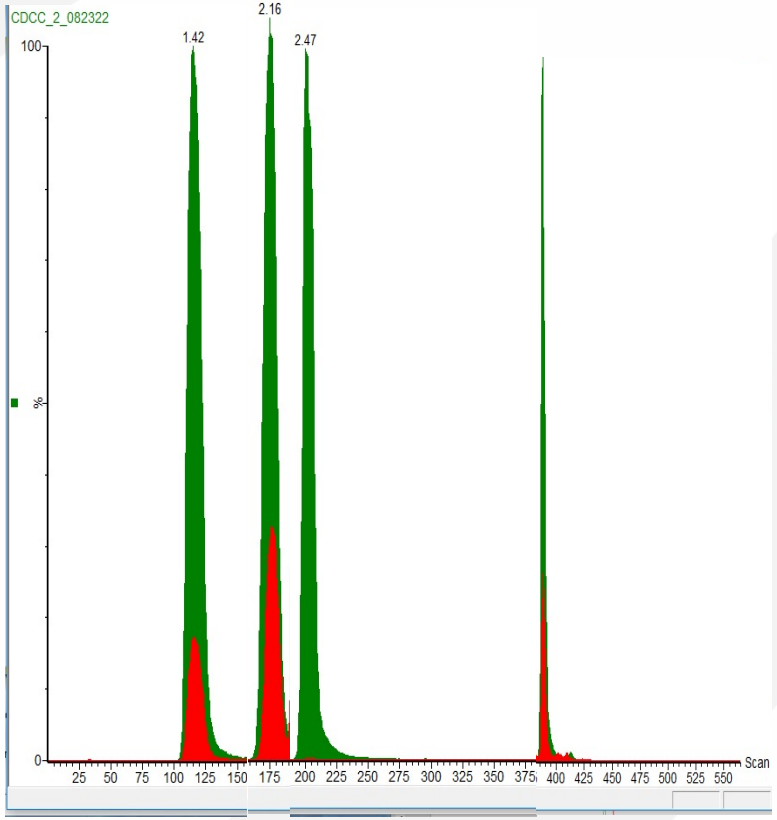
QC Samples

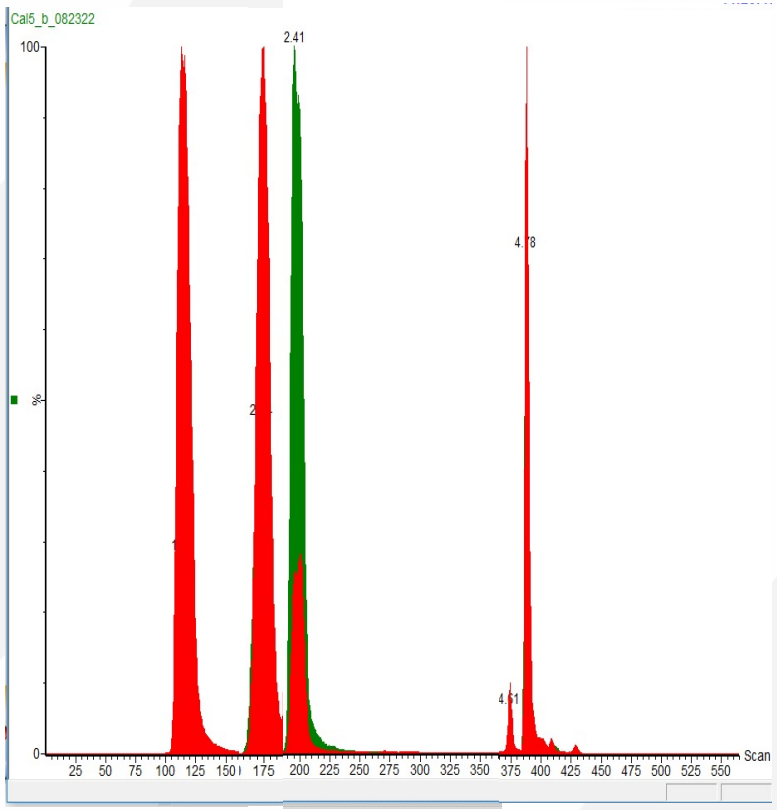
- CDC-A
- CDC-C
- In-house

Composite of Extracted Masses for Each Analyte and its IS

Intensity (%)







CDC- A

tCys
tHcy - 0 µM
Cysta
MMA - 2 µM

CDC- C

tCys
tHcy - 50 µM
Cysta
MMA - 20 µM

NENSP-High

tCys - 1000 µM
tHcy - 200 µM
Cysta - 20 µM
MMA - 200 µM

Analysis of tHcy & MMA Concentrations in CDC controls
NENSP vs CDC

tHcy (µmoles/L)				
Sample	**Enrichment**	**NENSP-C8 column**	**CDC Mean**	**NENSP-C18 column**
CDC-A	0	3.57	3.62	3.87
CDC-B	10	6.23	6.34	
CDC-C	50	18.19	16.71	20.00
CDC-D	100	30.70	32.23	
MMA (µmoles/L)				
CDC-A	2	1.83	1.4	2.09
CDC-B	5	4.09	3.08	
CDC-C	20	13.55	11.72	15.6
CDC-D	50	29.25	29.86	

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Limitations and Future Considerations

- The two-tier strategy offers the advantage of substantially reducing the burden of false positives
  - However, pyridoxine responsive forms of CBS may not have elevated level of tHcy at the time of screening
  - Cases with increased methionine but without the expected increase in homocysteine at the time of screening will not be detected
- Incorporate molecular methods for mutation analysis of the common pyridoxine responsive alleles
- It is debatable whether the pyridoxine responsive forms of CBS would be identified even when assays to measure homocysteine as a primary marker are utilized

Summary

- Demonstrated a high throughput 2nd tier LC-MS/MS assay to simultaneously measure four specific 2nd analytes using a single DBS for screening HCU
- Next steps:
  - Validate the method using NBS DBS samples
  - Establish the Reference Ranges
  - Analyze the data to define algorithms
- The two-tier strategy should offer the advantage of substantially reducing the burden of false positives

Acknowledgments

New England Newborn Screening Program

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Eileen Fitzgerald
Deborah Britton
All members of the Metabolic Lab

Centers for Disease Control

HCU Network America

BREAKING NEWS!

HCU Network America Announces the Recipient of their First Newborn Screening Research Grant.

August 31, 2022 - The New England Newborn Screening Program, an initiative of UMass Chan Medical School's Commonwealth Medicine division, received the award to explore the development of reference ranges for additional newborn screening markers for early detection of classical homocystinuria and remethylation disorders. The research, led by Devinder Kaur, PhD, assistant professor of pediatrics at UMass Chan, aims to establish normal reference ranges for total homocysteine, along with other analytes collected by healthy newborns during the 24-48 newborn screening period. This will support the development of algorithms that will incorporate information on a variety of other variables in the future. Dr. Kaur, who is leading the research, joined the New England Newborn Screening Program in 2017 as a senior scientist.

To read the full press release, visit https://bit.ly/HCUNBSGrant

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