Improving Newborn Screening for Homocystinuria & Related Disorders
Talking Points

Classical Homocystinuria (HCU) is an inborn error of the metabolism that affects an estimated 1 in 200,000 newborns in the US, though the number may be much higher since the usual Newborn Screening (NBS) protocols used in the US do not identify all HCU newborns. Once patients are diagnosed, they are treated with a combination of B6, folic acid, B12, betaine, medical formula, and usually a low protein diet. Patients who are undiagnosed and/or untreated are at high risk for progressive nearsightedness, lens dislocation, scoliosis, osteoporosis, strokes and blood clots. Since our organization to support patients with HCU was founded in 2016, we have frequently heard from patients (or their caregivers) that they were not identified by NBS and their HCU was diagnosed up to several years later, usually due to clinical symptoms. Experts have estimated that 20 to 50% of pyridoxine non-responsive HCU patients (who are more severely affected) are missed by current NBS approaches[1]. This is why we are asking you for help!

What's the problem? Why are so many patients missed?
Massachusetts led the way with NBS for HCU by adding a Guthrie bacterial inhibition assay for methionine (MET) to their NBS panel in 1968. Slowly many other states joined the ranks, but it wasn't until 2009, after the Newborn Screening Saves Lives Act of 2007, that HCU officially became part of the Recommended Uniform Screening Panel (RUSP). Between 1968 and 2009 many changes took place in NBS, and tandem mass spectrometry which includes MET became the new standard for screening. An assay for total homocysteine (tHCY), which would be a much more sensitive NBS test for HCU, has been used by a few NBS programs as a second-tier test when the primary newborn screening for MET showed an elevated level but the labor required, the complexity and the cost of the tHCY test has prevented its adoption as the primary newborn screen for HCU.

So that leads to the problem. MET is not as sensitive a biomarker as tHCY would be. In many cases, the baby who has HCU has not had an increased level of MET or a level sufficiently increased to be identified at a time of 24-48 hours of age, when the newborn screening blood specimen is collected. In addition, there is also no harmonized cut-off level for increased MET. Every state sets their own cut-offs, which range from 45 umol/L (hereinafter referred to as uM) to 100 uM across the US. There are known cases of HCU with just a mild elevation of MET who were detected in NBS because the program had a relatively low cut-off but would have been missed if born in a state with a higher cut-off. Higher MET cutoff levels are often set to minimize the number of false positives, but, unfortunately, are likely to lead to missing HCU.

Is there a better approach?
Experts in NBS for HCU in the US and internationally have recommended a revised process that includes a lower cut-off for MET (such as a range from 39 to 50 uM[1], depending on lab median) or a corresponding ratio of MET to Phenylalanine (PHE) or MET/PHE ratio, with a second-tier test for tHCY using the same dried blood spot [2]. This lowers the number of false positives and increases the likelihood of identifying newborns with HCU[3, 4]. There are several states now utilizing this approach in the US, and the second-tier test can be outsourced if needed. The CLIR tools (Collaborative Laboratory Integrated Reports) that are available free via Mayo Clinic are also being utilized by some states which enhance the specificity and ability to interpret NBS data[5]. The CDC is finalizing a second-tier assay for tHCY that will be made available to the state labs. Another step some states have taken is to perform a second newborn screen for MET (along with other analytes) a few weeks after birth, which may pick up additional cases but this would require routinely collecting a second screening specimen throughout the US and likely be vigorously opposed, as it has been since the beginning of NBS, because of its very large and expensive addition to NBS with a very low yield. Therefore, lowering the MET cutoff in NBS together with second tier testing for tHCY could greatly help to improve NBS for HCU.

Are there other disorders that could benefit from this two-tier approach?
The two-tier process described above can also help identify patients with severe MTHFR and specific cobalamin disorders, if the algorithm also includes a low cut-off for MET and MET/PHE ratio on the first screen, and for those that fall under the cut-off, a second-tier screen for tHCY. While severe MTHFR and cobalamin disorders are not primary conditions on RUSP, they are serious disorders and patients can benefit from early diagnosis. It is estimated that 1:100,000 to 150,000 babies are born each year in the US with these disorders. The algorithm may also utilize a lower cutoff for propionylcarnitine (C3-AC) and a second tier- test for methylcitric acid (MCA) and methylmalonic acid (MMA)

that may improve diagnosis of propionate disorders such as propionic acidemia, methylmalonic acidemia, and cobalamin C (cblC) deficiency, which are included in RUSP.

What can you do?

An important component of HCU Network America's mission is to support the advancement of diagnosis for HCU and related disorders. Since 2019, we have given testimony before the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC), spoken with leaders of many NBS programs, and engaged key opinion leaders in the realm of NBS. While we are making tremendous strides, we need your help to solve this issue.

For Newborn Screening Program or Lab leaders:

We are asking you to evaluate your current process and consider lowering your MET cut-off (if higher than 39 to 50 uM) and instituting second-tier testing for tHCY. The full process and rationale and benefits are described in the publications noted in footnote 2. Also, if laboratory resources are not available to implement a second-tier test for tHCY, HCU Network America has compiled a list of potential labs to which you could consider outsourcing. We have attached the list of potential labs for outsourcing as of June 1, 2020, and you can find the most up-to-date list at https://hcunetworkamerica.org/2-tier-nbs-labs/ which is under the Newborn Screening tab on our website. We will continue to update this list and encourage you to let us know if you are aware of any others we should add to the list.

We urge you to alter your process even if you believe you have not missed any HCU patients, as we know they are out there and that states do not have the reporting systems and "closed loops" to ensure that there is a full accounting of patients who may have been missed by NBS. Your willingness to take action proactively could lead to improved lives for many HCU patients and their families.

For Geneticists, Metabolic Clinicians, and others involved in treating HCU Patients

Can we ask you to contact your state or region's NBS lab and engage them in the conversation on NBS for HCU, and advocate for an improved process?

What should I talk to them about?

- What is your state's MET cut-off?
- Have you considered the benefits of using a lower MET cut-off (if higher than the recommended levels, e.g. 39 to 50 uM)
- Do the cut offs vary by weight, length or gestation period? Male or female?
- Does your state use a MET/PHE ratio?
- Are there other biomarker/analytes that you are looking at when diagnosing HCU?
- Have you considered using CLIR to aid in the interpretation of NBS results[5]
- Does your state have a routine 2nd NBS test?
    - When is the test administered?
    - Are the cut-offs the same?
- Does your state have a second-tier test for tHCY?
    - If so, what cut off do you use to trigger the second-tier test?
- Would you advocate to change the process to ensure we diagnose as many HCU patients at birth as possible?
    - Is there a committee on NBS within your state you need to work with to get support for this change?
- Would they like to talk to one of the experts who is implementing the recommended two-tier process?
    - If so, please contact HCU Network America's Executive Director, Danae Bartke, at dbartke@hcunetworkamerica.org
- Would they be willing to outsource the second-tier test if resources are not available?
    - If so, refer to the attached list or check our website at: https://hcunetworkamerica.org/2-tier-nbs-labs/ for an updated listing

[1]Morris AAM, Kožich V, Santra S et al. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency. *J Inherit Metab Dis*. 2017; 40: 49-74. https://doi.org/10.1007/s10545-016-9979-0

[2] Keller R, Chrastina P, Pavlíková M et al. Newborn screening for homocystinurias: Recent recommendations versus current practice. *J Inherit Metab Dis*. 2019; 42: 128– 139. https://doi.org/10.1002/jimd.12034

[3]Matern D, Tortorelli S, Oglesbee D et al. Reduction of false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: The Mayo Clinic experience (2004-2007). *J Inherit Metab Dis.* 2007; 30: 585-592

[4]Chace D, Hannon W. Impact of Second-Tier Testing on the Effectiveness of Newborn Screening. *Clin Chem.* 2010; 56: 1653-1655 https://doi.org/10.1373/clinchem.2010.153494

[5]Gavrilov DK, Piazza AL, Pino G et al. The Combined Impact of CLIR Post-Analytical Tools and Second Tier Testing on the Performance of Newborn Screening for Disorders of Propionate, Methionine, and Cobalamin Metabolism. Int J Neonat Screen. 2020; 6, https://www.mdpi.com/2409-515X/6/2/33

Newborn Screening Labs for Potential Outsourcing of Second-tier Test for Homocysteine

Lab Name: Biochemical Genetics Laboratory, Mayo Clinic
Lab Director: Dr. Dietrich Matern, Dr. Piero Rinaldo
Lab Phone Number: 1-800-533-1710
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If your lab can support outsourced second-tier testing for homocysteine, please forward their information to dbartke@hcunetworkamerica.org