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Multiplexing Homocysteine into First-Tier Newborn Screening Mass Spectrometry Assays Using Selective Thiol Derivatization

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Guest: Dr. Carla D. Cuthbert is the Chief of the Newborn Screening and Molecular Biology Branch (NSMBB) at CDC's National Center on Environmental Health.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the American Association for Clinical Chemistry. I'm Bob Barrett.

Newborn screening performed on dried blood spots collected from newborns in the first few days of life helps identify children with inherited metabolic disorders, in many cases before development of any symptoms. Early detection allows early intervention, which translates into improved patient outcomes. But what if newborn screening only detected 50% of patients with a certain condition? Would this still be considered an effective screen?

One strategy to ensure more affected patients are identified is to lower the threshold for a positive result, but this generates more false positives, leading to unnecessary worry for many families with unaffected children. Another strategy is to adopt a two-tier sequential approach, but this is a more cumbersome workflow and may require additional laboratory equipment and staff.

A new article appearing in the May 2023 issue of *Clinical Chemistry* has identified a potential solution to this problem. A researcher with extensive experience in newborn screening and biochemical genetics describes a new test method for the detection of homocystinuria using dried blood spots and lists its advantages over currently available methods.

In this podcast, we are pleased to be joined by one of the authors of that study. Carla D. Cuthbert, Ph.D. is the Chief of the Newborn Screening and Molecular Biology Branch at CDC's National Center on Environmental Health. Dr. Cuthbert previously served as the Director of the Biochemical Genetics Laboratory at the University of Miami from 2005 through 2009.

Dr. Cuthbert, could you tell us a little bit about homocystinuria and why it's so important to diagnose affected patients early in life?

Carla Cuthbert: Well, homocystinuria is an inborn error of metabolism that results from the inability to convert homocysteine to

cystathionine. Classical homocystinuria is caused by deficient activity of the enzyme called cystathionine betasynthase, which is a vitamin B6 dependent enzyme, and this deficiency results in elevated levels of methionine and homocysteine. Certain cobalamin deficiencies or vitamin B12 deficiencies can also result in elevated homocysteine levels as well.

Untreated, classical homocystinuria results in ocular issues such as ectopia lentis, dysfunction of the vascular system such as thromboembolism and vascular occlusions, skeletal deformities, developmental and intellectual disabilities, failure to thrive, and symptoms that are similar to Marfan syndrome. Many untreated individuals die by 25 years of age, and death is predominantly due to cerebrovascular or cardiovascular causes.

Given all of this, early presymptomatic diagnosis provides at-risk newborns with the opportunity for early treatment and medical intervention and gives the newborn the best chance of achieving improved outcomes and leading a healthier life.

Bob Barrett: Okay, so what are the characteristics of newborn screening test methods that are in use today?

Carla Cuthbert: As with other laboratory methods, it is important that the newborn screening test to be precise and accurate, and it should have high analytical sensitivity and specificity for the informative biomarker. This test, when used in the context of population screening, like newborn screening, should also have a high positive predictive value.

So, within a large normal population, it should effectively identify at-risk individuals while excluding those not at risk for this disorder. Newborn screening tests also need to be fit for purpose, since they are mostly run in public health laboratories with high test volumes and programs that require very high turnaround times.

So, they need to be on high throughput platforms with short analytical times. For efficiency's sake, it is advantageous if they could be multiplexed as opposed to being a standalone test. They should be capable of using small test volumes, and the informative biomarker really needs to be stable and measurable at appropriate levels in blood.

In short, the laboratory testing process must be robust and reliable, ensuring accuracy in measurements every day, for every test, on every sample from every baby.

Bob Barrett: How does this new test work?

Carla Cuthbert: Well, shortly after birth, dried blood spots are collected from newborns and shipped to a public health laboratory that

performs newborn screening. These are a series of routine tests that are aimed at identifying pre-symptomatic newborns with rare diseases that are often debilitating or deadly if undetected or left untreated.

So currently, in first tier newborn screening for homocystinuria using methionine, a sample of the newborn's dry blood spot is taken, mixed with isotopically labeled internal standard and solvents, then analyzed by a flow injection analysis tandem mass spectrometry platform. During this workflow, there are dozens of other diseases being screened, including homocystinuria, for that same newborn.

In our protocol, our protocol introduces a reduction step to release bound oxidized homocysteine, followed by derivatization that increases the sensitivity for homocysteine by several times. Furthermore, using our approach, the molecular weight and fragmentation pattern after derivatization are unique to homocysteine, eliminating the effect of previous interferences from endogenous compounds and internal standards that are used for measuring other biomarkers.

Bob Barrett: So, what makes it different from currently available tests?

Carla Cuthbert: Well, there are other tests for total homocysteine that use separation before analysis by mass spectrometry. These are currently used as second tier screening tests for babies with elevated methionine levels identified during first tier screening. So these assays have longer analysis times and are not as heavily multiplexed as our FIA-MS/MS assay.

One of the issues with a two-tier approach that uses methionine as the first-tier biomarker and homocysteine as a second-tier biomarker is that it can still miss newborns with homocystinuria, since only specimens that have elevated methionine are reflexed to second tier screening for confirmation. But methionine has the inherent problem of lower clinical sensitivity for homocystinuria.

In our assay, this is the first time that homocysteine was able to be multiplexed in the widely-used flow injection analysis tandem mass spectrometry newborn screening assay. With our testing approach, homocysteine, used as a first-tier biomarker, would have greater clinical sensitivity to more effectively detect newborns at risk for homocystinuria.

Bob Barrett: Well, finally, Dr. Cuthbert, what additional work needs to be done to move this test closer to clinical implementation?

Carla Cuthbert: Well, the next logical step would be for a state newborn screening laboratory to pilot the assay and provide feedback on its performance, its ability to detect at-risk newborns,

after running thousands of patient specimens. There has been really good traction towards this goal.

Bob Barrett: That was Dr. Carla Cuthbert from CDC's National Center on Environmental Health. She helped develop a new test method that improves the sensitivity of newborn screening for homocystinuria while simultaneously reducing the number of false positive results. She and her team published their findings in the May 2023 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.