Novel test could ensure newborns with a serious genetic disease receive essential treatment

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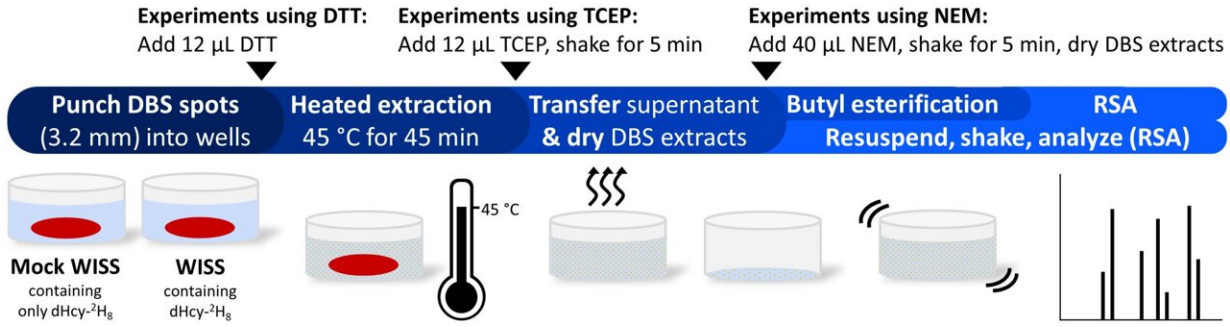

Sample preparation workflow used to investigate reducing and maleimide derivatizing agents. In all analyses, 3.2 mm DBSs of quality control materials, proficiency materials, and residual clinical specimens were punched into 96-well plates. Arrows (stemming from "Experiments using...") indicate where the first-tier screening method was modified to investigate reducing agents and maleimide derivatizing agents. DBSs were extracted using 100 µL WISS containing ISs for amino acids, acylcarnitines, nucleotides, homocystine (dHcy-$^{2}H_{8}$), and other biomarkers, along with formic acid and hydrazine hydrate. A mock WISS containing dHcy-$^{2}H_{8}$ along with formic acid and hydrazine hydrate was also used to investigate the presence of interferences from IS. In experiments using DTT, 12 µL of a DTT solution was added to the DBS containing WISS, then incubated for 45 min at 45°C. In experiments using TCEP, the DBS and WISS were incubated for 45 min at 45°C, then TCEP was added to the DBS extract solution and shaken for 5 min at room temperature. Sample extracts were then transferred, dried under nitrogen gas, and the DBS extracts were resuspended in 40 µL of a maleimide solution before being shaken for 5 min at room temperature. Maleimide treated extracts were then dried. Non-

butyl ester extracts were resuspended in mobile phase, then analyzed by FIA–MS/MS) In butyl ester-derivatized analyses, DBS extracts were resuspended in acidified butanol, then placed in an oven for 20 min. Butylated DBS extracts were then dried, resuspended in mobile phase, and then analyzed by FIA–MS/MS. Credit: *Clinical Chemistry* (2023). DOI: 10.1093/clinchem/hvad007

Scientists have developed a test that could greatly improve quality of life for infants with homocystinuria (HCU), a congenital disease that—if not treated early—causes serious complications. Research demonstrating the efficacy of this test was published today in *Clinical Chemistry*.

HCU impedes an infant's ability to metabolize the amino acid methionine, which is a component of many proteins, such as those found in breastmilk. This leads to a pathological increase in levels of methionine and another amino acid known as homocysteine, causing severe complications if left untreated. These complications range from eye and skeletal issues to vascular abnormalities and intellectual disabilities.

The good news is that early detection and treatment of HCU can prevent these complications. In light of this, since 2006, the U.S. Department of Health and Human Services has included HCU on the list of disorders for which newborns should be screened. However, current tests only measure levels of methionine, which are often still low when newborn screening occurs. As a result, it's estimated that these tests miss around 50% of HCU cases, which are then at high risk of going untreated.

In an effort to remedy this, a group of researchers led by Konstantinos Petritis, Ph.D., at the Centers for Disease Control and Prevention, has developed and validated a newborn screening test for HCU that works by

measuring homocysteine levels. In infants with HCU, homocysteine levels usually rise before methionine levels, and they almost always rise during the first few days of life when newborn screening is performed, making homocysteine a better early marker of this disease.

To evaluate the test's performance, Petritis's team used it to screen residual newborn screening specimens from infants who had already received diagnoses. One hundred of these samples were from healthy patients; 50 came from HCU-negative infants receiving total parenteral nutrition (TPN), which is given to premature babies in the NICU; and 2 samples were from HCU-positive patients.

The test successfully distinguished between the healthy and HCU-positive samples. It also accurately classed the TPN samples as HCU-negative, which is noteworthy because another problem with methionine tests for HCU is that they produce false positives in babies receiving TPN.

"Here we present the only flow injection analysis-tandem mass spectrometry first-tier newborn screening method that directly quantifies total homocysteine from dried blood spots," said Petritis.

"The ability to screen total homocysteine during first-tier newborn screening is a significant step toward reducing HCU false-negative rates, which will enable early identification and intervention to reduce HCU-associated morbidity and mortality."

More information: C Austin Pickens et al, Multiplexing Homocysteine into First-Tier Newborn Screening Mass Spectrometry Assays Using Selective Thiol Derivatization, *Clinical Chemistry* (2023). DOI: 10.1093/clinchem/hvad007