Homocystinuria is a metabolic condition in which the body is not able to properly process certain amino acids resulting in an abnormal accumulation of homocysteine and its metabolites in the blood and urine (NIH, 2018). Homocystinuria is usually due to genetic causes; however, homocystinuria could also be due to no-genetic causes. The non-genetic type of homocystinuria could also be due to non-genetic causes, including severe deficiency of vitamin B12, also known as cobalamin (Mudd et al, 2000).

***Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.

Policy

BCBSNC will provide coverage for homocystinuria testing when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When testing for homocystinuria is covered

1. Reimbursement is allowed for genetic counseling and is recommended prior to genetic testing for Homocystinuria.

2. Molecular genetic testing of CBS (encoding cystathionine β-synthase) gene is considered medically necessary for diagnosis and/or confirmation of Homocystinuria, and for carrier and prenatal testing.

3. Genetic testing for MTHFR, MTR, MTRR, and MMADHC genes is considered medically necessary if the following clinical conditions are met:
   - CBS testing has been done and is negative; AND
   - Patient has signs and symptoms of homocystinuria.

4. Reimbursement for newborn screening for homocysteine-related conditions is allowed in the following situations:
Testing of Homocysteine Metabolism Related Conditions AHS – M2141

A. For classic homocystinuria due to CBS deficiency by performing quantitative plasma amino acids analysis and/or plasma or urine total homocysteine analysis.
B. Testing for homocystinuria in dried blood spots
C. Testing for hypermethioninemia in dried blood spots.

5. Reimbursement is allowed for repeat dried blood specimen submitted to the newborn screening program, or a quantitative plasma amino acid analysis and analysis of plasma total homocysteine if the initial screening test result exceeds the cut-off level of methionine.

6. Reimbursement for a Pyridoxine (B6) Challenge test is allowed to diagnose phenotype variants of classic homocystinuria due to cystathionine β-synthase (CBS) deficiency.

7. Reimbursement for total homocysteine testing in plasma and dried blood spots is allowed in patients with suspected CBS deficiency and for monitoring therapy.

When Testing for homocystinuria is not covered

Reimbursement is not allowed for plasma free homocysteine testing.

Policy Guidelines

Background
Homocysteine (Hey), a naturally occurring intermediary amino acid, is involved in multiple metabolic pathways, including the transsulfuration pathway and methionine (Met) metabolism. Classical homocystinuria, resulting in an accumulation of Hey and its metabolites in the blood and urine, is due to genetic mutations in cystathionine-β-synthase (CBS), the enzyme in the rate-limiting step of the transsulfuration pathway (Zhu, Blake, Chan, Pearson, & Kang, 2018). CBS is dependent on a pyridoxine (also known as vitamin B6). If this enzyme is blocked, it limits transsulfuration of homocysteine and accumulation of both homocysteine and methionine because methionine concentration will be enhanced by remethylation. The disruption of methionine metabolic pathway shown in the figure 1 below [from (Zhu et al., 2018)] prevents homocysteine from being used properly, resulting in a buildup of homocysteine and toxic by-products in the blood, with excess homocysteine excreted in urine (NIH, 2018).
Homocystinuria due to CBS deficiency can cause eye problems, skeletal abnormalities, an increased risk for blood clots, and developmental delay. The exact incidence of CBS deficiency is unknown. It is estimated to be at 1:344,000 worldwide by Mudd and colleagues (Mudd et al., 1985). In European populations, it is predicted by molecular epidemiological studies to be between 1:6,400 and 1:20,500 (Gaustadnes, Ingerslev, & Rütiger, 1999; Janosík et al., 2009). Infants with homocystinuria due to CBS deficiency are asymptomatic at birth, but will slowly develop symptoms if left untreated. These symptoms are highly variable. Some patients exhibit mild symptoms of the disorder, while others may have potentially life-threatening complications. The phenotype of these patients mainly relates to pyridoxine-responsiveness. Those who respond well to pyridoxine treatment exhibit milder phenotype and a later onset than pyridoxine-unresponsive patients (Abbott, Folstein, Abbey, & Pyeritz, 1987; Mudd et al., 1985). Early detection and treatment is important in preventing or reducing the severity of the disorder. Screening for homocystinuria is frequently incorporated into state newborn screening programs (Rose & Dolan, 2012). A newborn blood spot specimen for hypermethioninemia will detect classic in some, but not all, affected individuals with homocystinuria (Sacharow, Picker, & Levy, 2004).

Biochemical features of homocystinuria (Sacharow et al., 2004) include:
- markedly increased concentrations of plasma homocysteine, total homocysteine, homocysteine-cysteine mixed disulfide, and methionine
- increased concentration of homocysteine in urine

Figure 1: Homocysteine is a common metabolite linked to multiple metabolic pathways, including methionine/S-adenosyl methionine (SAM) metabolism, transsulfuration, and desulfuration. Figure taken from (Zhu et al., 2018).
Testing of Homocysteine Metabolism Related Conditions AHS – M2141

- reduced cystathionine β-synthase (CBS) enzyme activity

Classical biochemical findings establishing the diagnosis are summarized in the following table (Sacharow et al., 2004)

Cardinal Biochemical Findings that Establish the Diagnosis of Homocystinuria (Sacharow et al., 2004).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Specimen</th>
<th>Expected Findings</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total homocysteine (tHcy)</td>
<td>Plasma</td>
<td>Neonate with homocystinuria</td>
<td>Untreated older individual with homocystinuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 to &gt;100 µmol/L</td>
<td>&gt;100 µmol/L</td>
</tr>
<tr>
<td>Methionine (on amino acid analysis)</td>
<td>Plasma</td>
<td>200-1500 µmol/L (3-23 mg/dL)</td>
<td>&gt;50 µmol/L (&gt;0.7 mg/dL)</td>
</tr>
</tbody>
</table>

**Analytical Validity**

This concentration of homocysteine in blood plasma (tHcy) is the primary clinical analyte measured to diagnose homocystinuria. For example, a study using LC-MS calculated their limits of detection and quantification to be 0.06 µmol/L and 0.6 µmol/L respectively (Nelson, Pfeiffer, Sniegoski, & Satterfield, 2003). Another study using GC-MS found a detection limit of 0.4 µmol/L as well as intra- and inter-run variations of 5% and 8% respectively. Furthermore, this method was found to compare well with the LC-MS-MS method; the GC-MS method had a mean difference of -0.4 µmol compared to the LC-MS-MS method (Belkhiria et al., 2007). The FPIA method was found to compare favorably to the HPLC and MS approaches (5% imprecision with -2% to 3% bias), so it may be a more practical option if the more precise approaches are not available; however, this study only measured levels up to 45 µmol/L whereas severe homocystinuria can exceed 100 µmol/L (Nexo et al., 2000).

**Clinical Validity and Utility**

The diagnosis of classic homocystinuria (caused by CBS deficiency) is established by measurement of plasma homocysteine or tHcy. The normal level is <15 uMol/L whereas a newborn with homocystinuria is expected to measure out at >50 uMol/L and an older, untreated individual is expected to measure at >100 uMol/L. A measurement of methionine in plasma can corroborate a diagnosis as the metabolic pathway involves a buildup of methionine in addition to the buildup of homocysteine (Sacharow et al., 2004).

Free homocysteine (which composes about 15-25% of total homocysteine levels (Rosenson, 2018)) testing is unnecessary as total homocysteine testing already includes all forms of homocysteine. Total homocysteine testing converts all forms of homocysteine into a single species so the amount of free homocysteine is already included in the total homocysteine measurement (Ueland et al., 1993).

The detection of biallelic pathogenic variants in cystathionine β-synthase can substantiate the diagnosis of homocystinuria (Sacharow et al., 2004). Two phenotypic variants are recognized in homocystinuria caused by cystathionine β-synthase, B6-responsive and B6-non-responsive homocystinuria. The pyridoxine (B6) challenge test is performed to determine the variant and if vitamin B6 therapy will be beneficial (Sacharow et al., 2004). Testing for homocystinuria usually involves biochemical testing in urine and/or molecular genetic testing for known mutations. The molecular genetic testing could be done using a single gene or multi-gene panels which could
Testing of Homocysteine Metabolism Related Conditions AHS – M2141

include sequence analysis, deletion/duplication analysis and/or other non-sequencing-based tests. Since homocystinuria usually involves CBS deficiency, the activity of CBS enzyme could be performed in cultured fibroblasts when genetic tests are inconclusive. However, enzymatic testing for CBS deficiency is no longer available in USA (Sacharow et al., 2004).

Homocystinuria due to genetic causes is inherited in an autosomal recessive pattern. Many different forms of homocystinuria can occur, and signs and symptoms vary depending on the gene mutation. CBS (Cystathionine Beta-Synthase) gene mutations cause the most common form of homocystinuria. This mutation is referred to as “classic” homocystinuria and is also known as CBS deficiency. Gene mutations in MTHFR, MTR, MTRR, and MMADHC can result in homocystinuria as well. The MTHFR, MTR, and MTRR genes all revolve around the remethylation pathway of Hcy while the MMADHC gene plays a role in Vitamin B12 metabolism (Froese et al., 2015; W. Wang, Jiao, Wang, Sun, & Dong, 2016).

Methylene Tetrahydrofolate Reductase (MTHFR) mutations are of interest. This enzyme catalyzes the reduction of 5,10 methylenetetrahydrofolate to 5,10 methyltetrahydrofolate, which is the methyl donor for the conversion of Hcy to methionine (Met). Failure of this enzyme (<20% of normal levels) leads to increased Hcy and increased Met as well as the symptoms detailed above (Long & Goldblatt, 2016).

The two most common mutations in the MTHFR gene are 677T (changed from a C nucleotide) and 1298C (changed from an A). Both mutations can be heterozygotic or homozygotic, and both can lead to loss of enzymatic function (Gonzales, Yu, & Shiao, 2017). The 677 mutation is more severe; its homozygous form (TT) was found to result in up to 70% loss of enzymatic function whereas its heterozygous form of CT was found to result in a maximum of 35% loss (Frosst et al., 1995). The 1298 mutation also resulted in loss of enzymatic function; 30% and 15% for its homozygous and heterozygous forms respectively (Weisberg, Tran, Christensen, Sibani, & Rozen, 1998). However, it is possible that dietary factors (notably low levels of folate or Vitamin B12) influence tHcy levels more than genetic factors. A study covering 452 young adults found the total genetic contribution (i.e. genetic polymorphisms) to the tHcy variance to be only 9% compared to 35% that could be attributed to dietary factors. The only polymorphism found to have a significant effect on tHcy levels was the 677T mutation, which interacted with low folate levels to produce a high tHcy phenotype. Compared to the authors’ earlier studies of genetic influence on tHcy levels, the younger cohort’s genetic contribution on tHcy levels was measured out to be higher than the older’s cohort’s (9% compared to 7% for the older cohort’s), and the authors suggest that genetic influence on tHcy levels are more pronounced during early life and environmental factors are more influential as time passes. (Gaughan et al., 2001; Harmon et al., 1999; Kluijtmans et al., 2003).

Applicable Federal Regulations
A search of the FDA Device database on 10/31/2018 for “homocysteine” yielded 30 results, last updated 07/31/2012. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ‘88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

On May 13, 2011, the FDA approved the Invader MTHFR 677 created by Hologic, Inc. The Invader MTHFR 677 is an in-vitro diagnostic test intended for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10-methylenetetrahydrofolate reductase (MTHFR) gene in isolated genomic DNA obtained from whole blood Potassium EDTA samples from patients with suspected thrombophilia.

On April 25, 2011, the FDA approved the Invader MTHFR 1298 created by Hologic, Inc. The Invader MTHFR 1298 test is an in vitro diagnostic test intended for the detection and genotyping of a single point mutation (A to C at position 1298) of the human 5,10-methylenetetrahydrofo late
An Independent Licensee of the Blue Cross and Blue Shield Association

Testing of Homocysteine Metabolism Related Conditions AHS – M2141

reductase (MTHFR) gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia.

On April 22, 2010, the FDA approved the eSensor Thrombophilia Risk Test on XT-8 System created by Osmetech Molecular Diagnostics. The MTHFR-specific portion is as follows: The eSensor MTHFR Genotyping Test is an in-vitro diagnostic for the detection and genotyping of point mutations (C to T at position 677) and (A to C at position 1298) of the human 5,10-methylenetetrahydrofolate reductase (MTHFR) gene in isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the eSensor XT-8 System.

On October 11, 2007, the FDA approved the Verigene System created by Nanosphere Inc. The MTHFR-specific portion is as follows: The Verigene MTHFR Nucleic Acid Test is an in vitro diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10 methylenetetrahydrofolate reductase gene (MTHFR) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene System (FDA, 2018).

Policy Statement(s)

Guidelines and Recommendations:

American College of Medical Genetics: Newborn Screening ACT Sheet

ACMG recommends quantitative plasma amino acids testing to determine if there is an increased homocysteine and methionine as in classical homocystinuria or only increased methionine like in the other disorders. Also, plasma homocysteine analysis will show markedly increased homocysteine in classical homocystinuria and normal or only slightly increased homocysteine in the other disorders. The urine homocysteine will be significantly increased in classical homocystinuria ("Newborn Screening ACT Sheet [Increased Methionine] Homocystinuria (CBS Deficiency)," 2010).

In the Confirmatory Algorithms for Methionine, ACMG indicates that increased methionine and increased total homocysteine are indicative of homocystinuria due to CBS deficiency ("Methionine Elevated or Decreased," 2009).

Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines: (Huemer et al., 2015)

Authors recommend newborn screening for CBS deficiency by detecting elevated methionine, methionine-to-phenylalanine ratio and/or total homocysteine in dried blood spots. They recommend increasing specificity by analyzing total homocysteine as a second-tier marker and calculating Met/tHcy ratio.

Newborn screening for the cblD-Hcy, CblE, and cblG defects, and for MTHFR deficiency could be possible by measuring methionine and methionine-to-phenylalanine ratio in dried blood spots followed by analysis of total homocysteine as a second-tier marker. However, authors believe that the efficacy and feasibility of screening for these disorders is largely unknown (Huemer et al., 2015)

European Network and Registry for Homocystinuria and Methylation Defects (E-HOD) (Morris et al., 2017):

This guideline was written as part of the European network and registry for homocystinuria and methylation defects (E-HOD) and it provided practical guide to recognition, diagnosis and management of CBS deficiency. The guideline presented 41 separate recommendations based on literature review by Guideline Development Group. The authors admitted that the quality of the evidence that they found was poor and many of their recommendations were grade D. However, the
highest recommendation was given to measuring the plasma total homocysteine concentrations in any patient whose signs and symptoms strongly suggest the diagnosis.

For the biochemical diagnosis, a total homocysteine test is recommended as “the frontline test” for the diagnosis of CBS deficiency. Plasma free homocysteine is only detectable at tHcy concentrations above 50-60 µmol/L; its measurement is not particularly sensitive or even reproducible and is not recommended. Untreated patients with a CBS deficiency typically have tHcy concentrations above 100 µmol/L, and a diagnosis is likely if an elevated tHcy is found along with a high or borderline high plasma Met concentration. Further information such as low plasma cystathionine concentration or increased Met:Cystathionine ratio can support a diagnosis. tHcy measurement using dried blood spots can be done if plasma processing is not possible.

E-HOD recommends confirming CBS deficiency by measuring cystathionine synthase activity in fibroblasts or plasma and/or by mutation analysis of CBS gene. The gold standard for confirming CBS deficiency is determination of cystathionine production of Hcy and serine in cultured fibroblasts. Either the enzyme or DNA can be analyzed, and if one method does not confirm a diagnosis the other method should be done. The grade of this recommendation is B-C. Despite technical pitfalls of DNA testing, E-HOD recommends a molecular genetic analysis of the CBS gene for the confirmation of CBS deficiency and for carrier and prenatal testing (grade B). For the prenatal diagnosis, the molecular analysis is a preferred technique during the first trimester of pregnancy. If the mutations are known in the family, enzyme analysis can be performed in cultured amniocytes, but not in chorionic villi. Preimplantation analysis also could be done (grade C-D).

For the newborn screening, it is recommended to increase specificity of methionine (Met) testing by using total homocysteine (tHcy) as a second marker and calculating Met/tHcy ratio (grade C). Several other metabolic disorders can cause an increased Met concentration and the exact sensitivity of detecting Met in newborns with a CBS deficiency is unknown. Although the median Met concentration of CBS deficient patients is far greater than the median of a healthy neonate (103 µmol/L compared to 20 µmol/L), individual Met values may still vary.

Screening for the family members at risk is recommended by measuring total homocysteine, molecular genetics could be also done in exceptional cases (grade D).

Monitoring of total homocysteine, amino acids, folate and vitamin B12 is recommended in all patients during therapy. The frequency of the monitoring is variable case by case (due to severity, treatment plan, age, etc). The targeted concentration ranges for total plasma homocysteine are proposed to be <50 mmol/L in pyridoxine-responsive patients and at <11 mmol/L free homocysteine (about 120 mmol/L total homocysteine) in pyridoxine-unresponsive patients (Morris et al., 2017).

**Billing/Coding/Physician Documentation Information**

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

*Applicable service codes: 81291, 81401, 81406, 81479, 82136, 82139, 82615, 83090, 83621, 84207, 96040, S0265*

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.
Testing of Homocysteine Metabolism Related Conditions AHS – M2141

Scientific Background and Reference Sources


Testing of Homocysteine Metabolism Related Conditions AHS – M2141


University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.


Testing of Homocysteine Metabolism Related Conditions AHS – M2141


Avalon review 12/2018

Specialty Matched Consultant Advisory Panel review 02/2020

Policy Implementation/Update Information

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1/19</td>
<td>New policy developed. CBSNC will provide coverage for homocystinuria testing when it is determined to be medically necessary because the medical criteria and guidelines are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (an)</td>
</tr>
<tr>
<td>4/16/19</td>
<td>Policy renamed from Homocystinuria to Testing of Homocysteine Metabolism Related Conditions. Avalon Annual Review: Updated description, background, federal regulations, guidelines, and evidence-based scientific references. Added clinical criteria: “For symptomatic patients (i.e. having elevated urine and/or serum homocysteine levels) that test negative for CBS classic homocystinuria OR for patients with a first-degree relative positive for known variants of MTHFR that cause homocystinuria, genetic testing for known variants of MTHFR is considered medically necessary.” Added clinical criteria that plasma free homocysteine testing does not meet medical necessity criteria based on 2017 E-HOD guidelines. Combined the newborn screening for homocystinuria and hypermethioninemia into one statement. (an)</td>
</tr>
<tr>
<td>10/29/19</td>
<td>Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (gm)</td>
</tr>
<tr>
<td>02/11/20</td>
<td>Reviewed by Avalon 4th Quarter CAB. Coding section updated. (eel)</td>
</tr>
<tr>
<td>03/10/20</td>
<td>Specialty Matched Consultant Advisory Panel 02/19/2020. No change to policy statement. (eel)</td>
</tr>
</tbody>
</table>

Medical policy is not an authorization, certification, explanation of benefits or a contract. Benefits and eligibility are determined before medical guidelines and payment guidelines are applied. Benefits are determined by the group contract and subscriber certificate that is in effect at the time services are rendered. This document is solely provided for informational purposes only and is based on research of current medical literature and review of common medical practices in the treatment and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.