

Testing of Homocysteine Metabolism-Related Conditions

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| Policy Number: AHS – M2141 – Testing of Homocysteine Metabolism-Related Conditions | Prior Policy Name and Number, as applicable: <ul style="list-style-type: none"> AHS – G2096 – Homocysteine Testing AHS – M2141 – Homocystinuria |
| Initial Presentation Date: 12/12/2016 | |
| Revision Date: 12/02/2020 | |

I. Policy Description

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, 2016, 2018a). Homocystinuria is usually due to genetic causes; however, homocystinuria could be also due to non-genetic causes, including severe deficiency of vitamin B12, also known as cobalamin (Mudd et al., 2000).

II. Related Policies

| Policy Number | Policy Title |
|---------------|---|
| AHS-G2014 | Vitamin B12 And Methylmalonic Acid Testing |
| AHS-G2035 | Prenatal Screening |
| AHS-G2050 | Cardiovascular Disease Risk Assessment |
| AHS-M2041 | Venous and Arterial Thrombosis Risk Testing |

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request

1. Genetic Counseling **MEETS COVERAGE CRITERIA** and is recommended prior to genetic testing for Homocystinuria.

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2. 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* **MEETS COVERAGE CRITERIA**.
3. Newborn screening for homocysteine-related conditions **MEETS COVERAGE CRITERIA** in the following situations:
 - 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.
4. A repeat dried blood specimen submitted to the newborn screening program, or a quantitative plasma amino acid analysis and analysis of plasma total homocysteine **MEETS COVERAGE CRITERIA** if the initial screening test result exceeds the cut-off level of methionine.
5. Pyridoxine (B6) Challenge test **MEETS COVERAGE CRITERIA** to diagnose phenotype variants of classic homocystinuria due to cystathionine β -synthase (CBS) deficiency.
6. Total homocysteine testing in plasma **MEETS COVERAGE CRITERIA** in patients over 18 years with suspected CBS deficiency with homocystinuria and for monitoring therapy in those with confirmed CBS.
7. Plasma free homocysteine testing **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Homocysteine (Hcy), a naturally occurring intermediary amino acid, is involved in multiple metabolic pathways, including the transsulfuration pathway and methionine (Met) metabolism. Classical homocystinuria, which results in an accumulation of Hcy and its metabolites in the blood and urine, is due to genetic mutations in cystathionine- β -synthase (CBS). CBS is the enzyme responsible for the rate-limiting step of the transsulfuration pathway and is dependent on pyridoxine (vitamin B6) (Zhu, Blake, Chan, Pearson, & Kang, 2018). If this enzyme is blocked, the transsulfuration of Hcy and the accumulation of both Hcy and Met will be limited because Met concentration will be enhanced by remethylation. The disruption of the Met metabolic pathway, as shown in Figure 1 below (Zhu et al., 2018), prevents Hcy from being used properly; this creates in a buildup of Hcy and toxic by-products in the blood, with excess Hcy excreted in urine (Mazaheri, Mostofizadeh, & Hashemipour, 2017; NIH, 2018a).

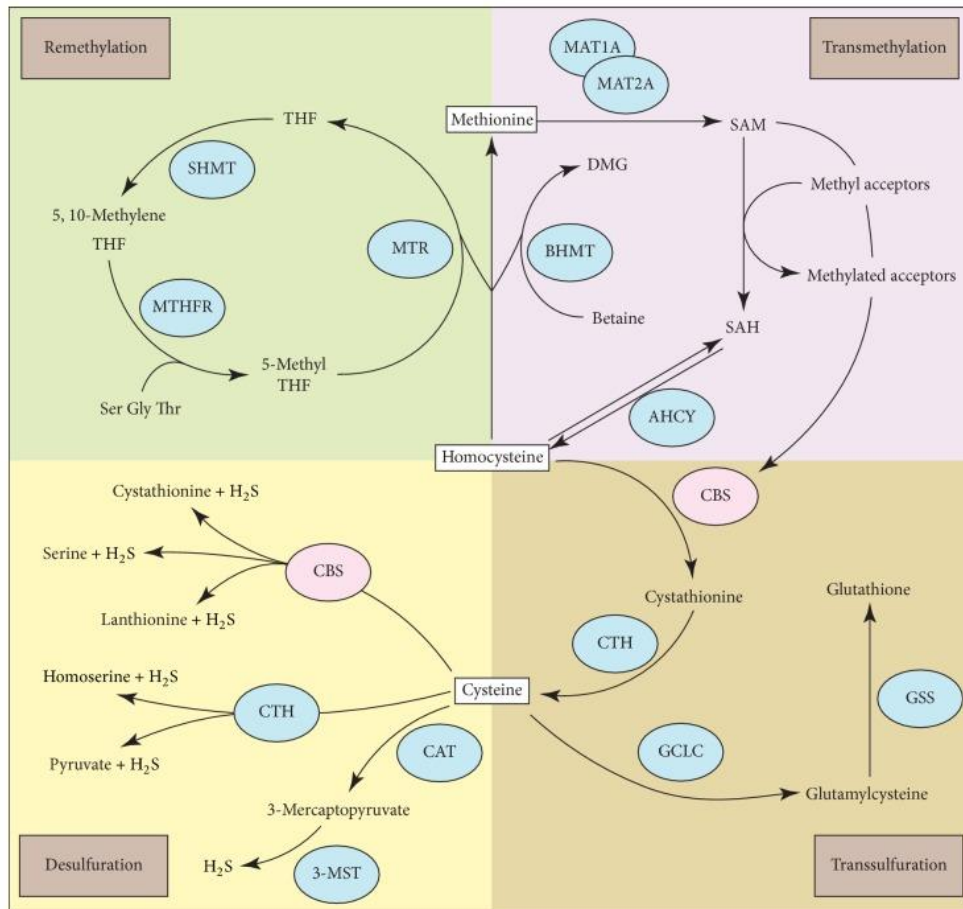


Figure 1: Homocysteine is a common metabolite linked to multiple metabolic pathways, including methionine/S-adenosyl methionine (SAM) metabolism, transsulfuration, and desulfuration. Figure taken from (Zhu et al., 2018).

Homocystinuria due to CBS deficiency can cause eye problems, skeletal abnormalities, an increased risk for blood clots, and developmental delay. Homocystinuria may also generate white matter abnormalities in the brain, potentially mimicking other disorders on computed tomography (CT) and magnetic resonance imaging (MRI) scans, including leukoencephalopathy (Ismayilova, MacKinnon, Mundy, & Fallon, 2019; Li, Barshop, Feigenbaum, & Khanna, 2018).

The exact incidence of homocystinuria due to CBS deficiency is unknown. In 1985, the incidence was estimated to be at 1:344,000 worldwide (Mudd et al., 1985). However, the National Institutes of Health (NIH) is now estimating these rates to be much higher at 1:150,000 worldwide, 1:200,000-300,000 in the United States, and 1:1,800 in Qatar (NIH, 2018b). In European populations, incidence rates have been predicted by molecular epidemiological studies to be between 1:6,400 and 1:20,500 (Gaustadnes, Ingerslev, & Rütiger, 1999; Janosík et al., 2009). Higher prevalence in the MENA (Middle East and North Africa) region could be attributed to high consanguinity in those communities (Al-Sadeq &

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Nasrallah, 2020). Infants with homocystinuria due to CBS deficiency are asymptomatic at birth, but they will slowly develop symptoms if left untreated. These symptoms are highly variable. Affected patients may exhibit mild symptoms of the disorder while others may develop potentially life-threatening complications. Symptoms depending on the population affected and type of CBS gene mutation can be as severe as including ectopia lentis, Marfanoid features, mental retardation, idiopathic infertility, osteoporosis, and severe premature atherosclerosis (Al-Sadeq & Nasrallah, 2020; Rosenson, Smith, & Bauer, 2020). The phenotype of these patients mainly relates to pyridoxine-responsiveness. Those who respond well to pyridoxine treatment exhibit a milder phenotype and a later onset than pyridoxine-unresponsive patients (Abbott, Folstein, Abbey, & Pyeritz, 1987; Mudd et al., 1985). Early detection and treatment are 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 homocystinuria due to CBS deficiency in some, but not in all affected individuals (Sacharow, Picker, & Levy, 2004).

According to Sacharow et al. (2004), the biochemical features of homocystinuria include:

- Markedly increased concentrations of plasma homocysteine, total homocysteine, homocysteine-cysteine mixed disulfide, and methionine
- Increased concentration of homocysteine in urine
- Reduced CBS enzyme activity

Classical biochemical findings establishing the diagnosis are summarized in the following table titled: Cardinal Biochemical Findings that Establish the Diagnosis of Homocystinuria (Sacharow et al., 2004).

| Analyte | Specimen | Expected Findings | | |
|-------------------------------------|----------|--|--|--|
| | | Neonate with homocystinuria | Untreated older individual with homocystinuria | Control |
| Total homocysteine (tHcy) | Plasma | 50 to >100 $\mu\text{mol/L}$ | >100 $\mu\text{mol/L}$ | <15 $\mu\text{mol/L}$ |
| Methionine (on amino acid analysis) | Plasma | 200-1500 $\mu\text{mol/L}$ (3-23 mg/dL) | >50 $\mu\text{mol/L}$ (>0.7 mg/dL) | 10-40 $\mu\text{mol/L}$ (0.2-0.6 mg/dL) |

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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* gene mutations cause the most common form of homocystinuria. This mutation is referred to as “classic” homocystinuria or CBS deficiency. Gene mutations in *MTHFR*, *MTR*, *MTRR*, and *MMADHC* can result in homocystinuria. 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; Wang, Jiao, Wang, Sun, & Dong, 2016).

Homocystinuria may also be associated with a diagnosis of methylmalonic acidemia; this is when the body cannot efficiently break down specific fats or proteins, which leads to a methylmalonic acid buildup in the blood. Methylmalonic aciduria and homocystinuria type C (cblC) is characterized by a vitamin B12 disorder initiated by a mutation in the *MMACHC* gene; symptoms of this disorder fall into several categories, including thromboembolic and neurological issues such as cognitive and psychiatric episodes (Collison et al., 2015).

Analytical Validity

This concentration of total homocysteine (tHcy) in blood plasma is the primary clinical analyte measured to diagnose homocystinuria. A study using liquid chromatography–mass spectrometry (LC-MS) calculated limits of detection and quantification of tHcy to be 0.06 $\mu\text{mol/L}$ and 0.6 $\mu\text{mol/L}$ respectively (Nelson, Pfeiffer, Sniegowski, & Satterfield, 2003). Another study using gas chromatography–mass spectrometry (GC-MS) found a detection limit of 0.4 $\mu\text{mol/L}$ 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). Fluorescence polarization immunoassay (FPIA) was found to compare favorably to the high performance liquid chromatography (HPLC) and MS approaches as well (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 $\mu\text{mol/L}$ whereas severe homocystinuria can exceed 100 $\mu\text{mol/L}$ (Nexo et al., 2000).

More recently, Concepción-Alvarez, Camayd-Viera, and Nuevas-Paz (2016) have validated a method to quantify Hcy in plasma samples via HPLC; Hcy levels were measured in a total of 46 patients. The authors have stated that HPLC was able to “identify and quantify Hcy without interferences,” and that the identified detection limit was 3.12 μM and quantification limit 6.25 μM (Concepción-Alvarez et al., 2016). This research has provided further validation for Hcy plasma testing in ailments where this amino acid is increased such as homocystinuria.

Methylmalonic acid and tHcy are commonly measured in both plasma samples and dried blood spots for the detection of Hcy-related conditions; de Sain-van der Velden et al. (2015) recently compared methylmalonic acid and tHcy levels via both dried blood spot and plasma concentration testing methods using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to determine which is the more efficient and accurate method. The authors note that the plasma assay performed better than the dried blood spot testing

method in most areas, but dried blood spot testing was superior for tHcy stability; further, a strong correlation of tHcy was found in both testing methods, ($y=0.46\pm 1.12$ ($r(2)=0.91$)), leading to the authors suggestion that tHcy testing in plasma can be replaced by tHcy in dried blood spots (de Sain-van der Velden et al., 2015).

Clinical Validity and Utility

A diagnosis of classic homocystinuria (caused by CBS deficiency) is established by measurement of tHcy. The normal level is $<15 \mu\text{Mol/L}$, whereas a newborn with homocystinuria is expected to measure at $>50 \mu\text{Mol/L}$; an older, untreated individual is expected to measure at $>100 \mu\text{Mol/L}$ (Sacharow et al., 2004). A measurement of Met in plasma can corroborate a diagnosis as the metabolic pathway involves a buildup of Met in addition to the buildup of Hcy (Sacharow et al., 2004). Free Hcy composes about 15-25% of tHcy levels. However, separate free Hcy testing is unnecessary as tHcy measurement already includes all forms of Hcy (Rosenson et al., 2020).

The detection of biallelic pathogenic variants in CBS can substantiate a diagnosis of classic homocystinuria (Sacharow et al., 2004). Two phenotypic variants are recognized in homocystinuria and are caused by CBS, 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 genetic testing for known mutations. The testing could be done using a single gene or multi-gene panels which may include sequence analysis, deletion/duplication analysis and/or other non-sequencing-based tests. Since homocystinuria typically involves CBS deficiency, the activity of the 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).

Methylene tetrahydrofolate reductase (*MTHFR*) mutations are of interest in homocystinuria. The *MTHFR* enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5,10-methyltetrahydrofolate, which is the methyl donor for the conversion of Hcy to Met. Failure of this enzyme ($<20\%$ of normal levels) leads to increased Hcy and Met, as well as the production of other symptoms associated with homocystinuria (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 677T 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 35% loss (Frosst et al., 1995). The 1298C 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

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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 cohorts genetic contribution on tHcy levels was measured out to be higher than the older cohort's (9% compared to 7% for the older cohort); further, 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).

Another study conducted by Gales et al. (2018) found that a mutation leading to *MTHFR* deficiency in the context of adult or adolescent onset could create a focal epilepsy presentation. This makes understanding this mutation found in homocystinuria critical to detect for early treatment, as the neuropsychiatric syndrome could be easily treated with a combination of vitamin B9, vitamin B12, and betaine (Gales et al., 2018).

A novel newborn screening method has been developed by researchers following a two-tier algorithm using a methionine (Met) to phenylalanine (Phe) ratio; data from 125,047 neonates was utilized to determine this accuracy of this method (Okun et al., 2017). It was reported that "Met to Phe ratio was found to be more effective for first sieve than Met, sorting out nearly 90% of normal samples. Only 10% of the samples would have to be processed by second-tier measurement of Hcy in dried blood spots" (Okun et al., 2017). This novel testing method resulted in 100% sensitivity and specificity for classical homocystinuria newborn screening (Okun et al., 2017).

V. Guidelines and Recommendations

The U.S. Department of Health and Human Services (HHS, 2018)

The Secretary of the U.S. Department of HHS has developed a recommended uniform screening panel for every universal newborn screening program; the amino acid disorder homocystinuria is recommended as a core condition for newborn screening, and the organic acid condition methylmalonic acidemia with homocystinuria is recommended as a secondary screening condition. Methylmalonic acidemia due to methylmalonyl-CoA mutase or cobalamin disorders is included as a core condition as well (HHS, 2018).

American College of Medical Genetics (ACMG): Newborn Screening ACT Sheet (ACMG, 2010)

ACMG recommends quantitative testing of plasma amino acids to determine increased levels of Hcy and Met; classical homocystinuria is characterized by increases in both Hcy and Met, while increased Met may be indicative of other disorders (ACMG, 2010). Also, plasma Hcy analysis will show increased Hcy in classical homocystinuria and normal or only slightly increased Hcy in the other disorders. The urine Hcy will be significantly increased in classical homocystinuria (ACMG, 2010).

In the Confirmatory Algorithms for Met, ACMG indicates that increased Met and increased tHcy are indicative of homocystinuria due to CBS deficiency (ACMG, 2009).

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Newborn Screening for Homocystinurias and Methylation Disorders: Systematic Review and Proposed Guidelines (Martina Huemer et al., 2015)

Authors recommend newborn screening for CBS deficiency by detecting elevated Met, methionine-to-phenylalanine ratio and/or tHcy in dried blood spots; specificity is increased by analyzing tHcy as a second-tier marker and calculating Met/tHcy ratio is also suggested (Martina Huemer et al., 2015)

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

Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency (M. Huemer et al., 2017)

M. Huemer et al. (2017) “strongly recommend measuring plasma total homocysteine in any patient presenting with the combination of neurological and/or visual and/or haematological symptoms, subacute spinal cord degeneration, atypical haemolytic uraemic syndrome or unexplained vascular thrombosis.” For a “valid, timely laboratory diagnosis,” the authors also add:

- “We strongly recommend that investigations in patients with a suspected remethylation disorder should start with the measurement of total homocysteine in blood. We recommend the blood sample for tHcy to be centrifuged within an hour and kept at +4° or frozen until analysis. Immunoassays or chromatographic methods are suitable for tHcy measurement. (Quality of the evidence: moderate)
- We strongly recommend against measuring free homocysteine instead of total homocysteine. (Quality of the evidence: moderate)
- We strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started. (Quality of the evidence: moderate)” (M. Huemer et al., 2017).

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); practical guides to recognition, diagnosis and management of CBS deficiency were provided. The guideline presented 41 separate recommendations based on a literature review by the Guideline Development Group. The authors admitted that the quality of the identified data was poor and many of their recommendations were grade D;

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however, the highest recommendation was given to measuring the plasma total homocysteine concentrations in any patient whose signs and symptoms strongly suggest the diagnosis (Morris et al., 2017).

For the biochemical diagnosis, a tHcy test is recommended as “the frontline test” for the diagnosis of CBS deficiency. Plasma free Hcy is only detectable at tHcy concentrations above 50-60 $\mu\text{mol/L}$; its measurement is not particularly sensitive or even reproducible and is, therefore, not recommended. Untreated patients with a CBS deficiency typically have tHcy concentrations above 100 $\mu\text{mol/L}$, 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. Finally, 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 Met testing by using 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 $\mu\text{mol/L}$ compared to 20 $\mu\text{mol/L}$), individual Met values may still vary.

Screening for family members at risk is recommended by measuring tHcy; molecular genetic testing may also be utilized in exceptional cases (grade D).

Monitoring of tHcy, 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 $<50 \mu\text{mol/L}$ in pyridoxine-responsive patients and at $<11 \mu\text{mol/L}$ free homocysteine (about 120 $\mu\text{mol/L}$ total homocysteine) in pyridoxine-unresponsive patients (Morris et al., 2017).

VI. State and Federal Regulations, as applicable

A search of the FDA Device database on 11/01/2020 for “homocysteine” yielded 30 results. 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-methylenetetrahydrofolate 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, 2020).

VII. Applicable CPT/HCPCS Procedure Codes

| Code Number | Code Description |
|-------------|--|
| 81291 | <i>MTHFR</i> (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C) |
| 82136 | Amino acids, 2 to 5 amino acids, quantitative, each specimen |

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| 82139 | Amino acids, 6 or more amino acids, quantitative, each specimen |
| 82615 | Cystine and homocystine, urine, qualitative |
| 83090 | Homocysteine |
| 83921 | Organic acid, single, quantitative |
| 84207 | Pyridoxal phosphate (Vitamin B-6) |
| 96040 | Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family |
| S0265 | Genetic counseling, under physician supervision, each 15 minutes |

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VIII. Evidence-based Scientific References

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