Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC—at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in one of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

While modified guidelines for identifying such families has been introduced, there are now laboratory approaches that may also aid in establishing this diagnosis in a subset of patients with HNPCC. These approaches have arisen from studies that revealed the underlying genetic defect in many, but not all, families diagnosed with HNPCC, namely defective DNA mismatch repair (MMR).

Current data suggest that 50-60% of HNPCC families will have a hereditary defect in a MMR gene as the underlying genetic cause (hereditary defective MMR). Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:

- 40% associated with a mutation in hMSH2
- 40% associated with a mutation in hMLH1
- 10% associated with a mutation in hMSH6
- 10% other (unknown)

The genes involved in the remaining cases of HNPCC not involving defective MMR (approximately 40%) are, at this time, unknown.

Once a germline mutation is identified in an affected family member, predictive testing is then available for at-risk relatives. Mutation carriers face a significantly increased risk of colorectal cancer, as well as increased risk for cancers of the endometrium, kidneys, bladder, stomach, small bowel, pancreas, and ovaries.

### Clinical

Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC—at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in one of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

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- 10% associated with a mutation in hMSH6
- 10% other (unknown)

The genes involved in the remaining cases of HNPCC not involving defective MMR (approximately 40%) are, at this time, unknown.

### Useful For

Predictive testing for HNPCC (hereditary defective MMR) when an hMLH1 mutation has been identified in a family member with colon cancer.

### Interpretation

An interpretive report will include specimen information, pedigree, assay information, and risk to develop HNPCC.

### Cautions

- The presence of a hMLH1 mutation in an affected family member must be documented prior to performing this test.
- We strongly recommend that patients undergoing predictive testing receive genetic counseling both prior to testing and after results are available.
Test Title: MLH1 Known Mutation
#83002

References
October;69(4):780-790
November;58(22):5248-5257

Method
DNA Sequencing, Southern Blot Analysis

Specimen Required: Specimen should include a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) with relevant clinical information and cytogenetics results, if available. Specimen must arrive within 72 hours of draw.

Draw 2 lavender-top (EDTA) tube(s) of whole blood (10 mL) or a yellow-top (ACD) tube(s) and send in the original VACUTAINER(S). Invert several times to mix blood. Forward unprocessed whole blood promptly at ambient temperature only.

Reference Values: An interpretive report will be issued.

NOTE: Assay performed using analyte-specific reagent. See "Analyte-Specific Reagents (ASR) - Mayo Medical Laboratories" in Special Instructions.

Analytic Time: 14 days
Days Set Up: Specimens received by Friday at 12:00 noon will be set up on the next run. This assay is run every Monday.
Fee: $148.30
CPT Code: 83891x1/DNA extraction
83912x1/Interpretation and report
Urinary porphobilinogen (PBG) is elevated during the acute phase of the neurologic porphyrias: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), and variegate porphyria (VP). Acute attacks may produce symptoms of cerebral dysfunction and damage (confusion, "psychiatric disease"), autonomic neuropathy, constipation, urinary retention, tachycardia, hypertension, and severe (sometimes chronic) abdominal pain. In the acute phase, these disorders can be life threatening. Between attacks patients have mild symptoms that may include "psychoneuroses." Several studies indicate that approximately 1 in 300 psychiatric patients have AIP.

The common symptoms of AIP, HCP, and VP mimic many other diseases, from acute appendicitis to acute schizophrenia. Acute episodes usually begin with or include abdominal discomfort ranging from mild cramping to severe pain that suggests a need for surgical intervention. These symptoms and outward signs are common to a variety of medical problems and diagnosis usually depends on timely, accurate laboratory testing. Frequently, surgical intervention can be avoided if the laboratory returns an elevated level for urinary PBG.

**Clinical**

Urinary porphobilinogen (PBG) is elevated during the acute phase of the neurologic porphyrias: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), and variegate porphyria (VP). Acute attacks may produce symptoms of cerebral dysfunction and damage (confusion, "psychiatric disease"), autonomic neuropathy, constipation, urinary retention, tachycardia, hypertension, and severe (sometimes chronic) abdominal pain. In the acute phase, these disorders can be life threatening. Between attacks patients have mild symptoms that may include "psychoneuroses." Several studies indicate that approximately 1 in 300 psychiatric patients have AIP.

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**Useful For**

First-line test for establishing a tentative diagnosis for acute neuropathic porphyria including AIP, HCP, and VP

**Interpretation**

- Increased urinary PBG excretion in the presence of suggestive clinical symptoms is consistent with a biochemical diagnosis of acute porphyria (AIP, VP, and HCP).¹
- Urine and fecal porphyrin analysis should be performed to confirm the diagnosis and to distinguish between AIP, HCP, and VP. A biochemical diagnosis of AIP can be confirmed by measurement of PBG deaminase activity. VP and HCP can be confirmed by measurement of fecal porphyrins.

**Cautions**

- Ideally, specimen collection should occur during the acute phase.
- PBG is susceptible to degradation at high temperatures, at pH below 7.0, and on prolonged exposure to light. Specimens should be frozen immediately following collection and protected from light.
- PBG may be more accurately assessed with a 24-hour urine collection (#8562 “Porphyrins, Quantitative, Urine”).

**Supportive Data**

Inter-assay and intra-assay coefficients of variation were 3.8% and 3.0% respectively, at a mean concentration of 0.34 mg/L and 4.2% and 3.3% respectively, at a mean concentration of 1.02 mg/L. Mean recovery of PBG was 102% at a concentration of 0.24 mg/L, 101% at a concentration of 1.18 mg/L, and 100% at a concentration of 2.15 mg/L.

**References**

Porphobilinogen, Quantitative, Random, Urine

Method

PBG in urine is quantified by liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis in positive ion mode. An internal standard (13C2-PBG) is added to 1.0 mL of acidified urine. PBG and 13C2-PBG are extracted from the specimen using an Oasis HLB column on a Gilson ASPEC Workstation. Separation of PBG from the specimen matrix is achieved by a reverse phase HPLC. PBG and the internal standard are monitored through their corresponding precursor and product ion pairs. The ion pair for PBG and 13C2-PBG are m/z 227 to m/z 210 and m/z 229 to m/z 212, respectively. (Ford RE, Magera MJ, Kloke KM, et al: Quantitative measurement of porphobilinogen in urine by stable-isotope dilution liquid chromatography-tandem mass spectrometry. Clin Chem 2001 September;47(9):1627-1632)

Specimen Required: 20 mL from a random urine collection. No preservative. Send specimen frozen in a plastic, 60-mL urine bottle. Protect specimen from light.

Reference Values: 0.0-0.5 mg/g Creatinine

Analytic Time: 2 days

Days Set Up: Monday through Friday

Fee: $81.30

CPT Code: 84110
The biosynthesis of cholesterol and subsequent conversion to other essential compounds is complex, involving many intermediates and just as many enzymes. Disorders that result from the deficiency of these enzymes lead to the build up of certain intermediates and curtail the formation of important biomolecules, which can lead to serious birth defects and possibly death. Examples include:

- **Smith-Lemli-Opitz (SLO) syndrome** (3 beta-hydroxyysterol-delta 7-reductase deficiency), an autosomal recessive disorder characterized by markedly increased 7-dehydrocholesterol (7-DHC) levels, microcephaly, growth and development retardation, dysmorphic facial features, limb abnormalities (especially syndactyly and polydactyly of the toes), and heart and kidney malfunctions.

- **Desmosterolosis** (3 beta-hydroxyysterol-delta 24-reductase deficiency), which has a similar phenotype to SLO and results in the build up of desmosterol, a structural isomer of 7-DHC.

- **Sitosterolemia**, a rare autosomal recessive disorder marked by elevation of the plant sterols sitosterol and campesterol. Tendon and tuberous xanthomas, as well as premature coronary artery disease, result from the hyperabsorption of these sterols from the intestine. Sitosterolemia is due to mutations in 2 adenosine 5'-triphosphate (ATP)-binding cassette (ABC) transporters, ABCG5 and ABCG8.

Investigation of possible desmosterolosis (3 beta-hydroxyysterol-delta 24-reductase deficiency), sitosterolemia, and SLO syndrome (3 beta-hydroxyysterol-delta 7-reductase deficiency)

- Patients with sitosterolemia typically have campesterol values >40 ug/mL and sitosterol values >80 ug/mL.

- A quantitative report of the patient's sterol profile and a Biochemical Genetics consultant's interpretation will be provided for each specimen.

Cautions

Reference ranges were derived using fasting specimens from healthy individuals. Sitosterol and campesterol values may be mildly elevated in individuals whose diets include foods with high concentrations of plant sterols, such as some vegetable oils and infant formulas.

References


Method

The plasma specimen is hydrolyzed with 2% KOH/ethanol for 1 hour at 65 degrees C. The compounds are then extracted with hexane, followed by evaporation to dryness under nitrogen. Using 50:50 BSTFA:pyridine, the sterols are derivatized to generate trimethylsilyl ethers. The derivatized specimens are then transferred to gas chromatography sampling vials and 2.0 uL is injected onto a HP-5 capillary column. Selected ion-monitoring electron impact gas chromatography-mass spectrometry (GC-MS) is used to quantitate desmosterol, lathosterol, campesterol, and sitosterol in the reference range. Quantitative analysis in specimens with increased sterol concentrations (>10 ug/mL by GC-MS) is accomplished using gas chromatography-flame ionization detection (GC-FID) with epicoprostanol as the internal standard. (Kelley RI: Diagnosis of Smith-Lemli-Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. Clin Chim Acta 1995;236:45-58)
Specimen Required: Draw blood in a lavender-top (EDTA) tube(s) from a 12-hour fasting patient. Spin down, promptly separate plasma from cells, and send 0.4 mL of EDTA plasma frozen in plastic vial.

**NOTE:** 1. PATIENT’S AGE AND SEX ARE REQUIRED ON REQUEST FORM FOR PROCESSING.

Reference Values:
- DESMOSTEROL: 0.0-5.0 µg/mL
- LATHOSTEROL: 0.0-7.0 µg/mL
- CAMPESTEROL: 0.0-7.0 µg/mL
- SITOSTEROL: 0.0-5.0 µg/mL

Analytic Time: 2 days
Days Set Up: Varies
Fee: $115.00
CPT Code: 82542
N-methylhistamine (NMH) is the major metabolite of histamine, which is produced by mast cells. Increased histamine production is seen in conditions associated with increased mast-cell activity, such as allergic reactions, but also in mast-cell proliferation disorders, in particular mastocytosis. Mastocytosis is a rare disease. Its most common form, urticaria pigmentosa (UP), affects the skin and is characterized by multiple persistent small reddish-brown lesions that result from infiltration of the skin by mast cells. Systemic mastocytosis is caused by the accumulation of mast cells in other tissues and can affect organs such as the liver, spleen, bone marrow, and small intestine. The mast-cell proliferation in systemic mastocytosis can be either benign or malignant. In children, benign systemic mastocytosis tends to resolve over time, while in most, but not all adults, the disease is progressive. Systemic mastocytosis may or may not be accompanied by UP. Patients with UP or systemic mastocytosis can have symptoms ranging from itching, flushing, gastrointestinal distress, bone pain, and headaches, to anaphylactic shock.

Diagnosis of mastocytosis is made by bone marrow biopsy; however, patients with systemic mastocytosis usually exhibit elevated levels of NMH. Other biochemical markers include 11-beta prostaglandin F2 alpha, a metabolite of prostaglandin D2 (11 Beta-Prostaglandin F2 Alpha), and tryptase, alpha or beta (Tryptase).

Increased concentrations of urinary NMH are consistent with UP, systemic mastocytosis, or mast-cell activation. Because of its longer half-life, urinary NMH measurements have superior sensitivity and specificity than histamine, the parent compound. However, not all patients with systemic mastocytosis or anaphylaxis will exhibit concentrations outside the reference range, and healthy individuals may occasionally exhibit values just above the upper limit of normal.

The extent of the observed increase in urinary NMH excretion is correlated with the magnitude of mast-cell proliferation and activation. UP patients, or patients with other localized mast-cell proliferation and activation, show usually only mild elevations, while systemic mastocytosis and anaphylaxis tend to be associated with more significant rises in NMH excretion (2-fold or more). There is, however, significant overlap in values between UP and systemic mastocytosis, and urinary NMH measurements should not be relied upon alone in distinguishing localized from systemic disease.

Up to 25% variability in spot-urine excreted levels may be observed, making 24-hour urine collections preferable for cases with borderline results.

Children have higher NMH levels than adults. Adult levels are reached by the age of 16.
Cautions

- Individuals who are taking monoamine oxidase inhibitors (MAOIs) or aminoguanidine will have increased levels of NMH; results from patients on MAOIs are uninterpretable.
- While an average North American diet has no effect on urinary NMH levels, mild elevations (around 30%) may be observed on very histamine-rich diets. This problem is more pronounced if spot urine specimens rather than 24-hour urine specimens are used and the spot urine specimen is collected following a histamine-rich meal.
- NMH may be lowered in individuals who are receiving drugs that inhibit diamine oxidase.
- NMH levels may be depressed in individuals who have a polymorphism in the histamine-N-methyl transferase gene, which encodes the enzyme that catalyzes NMH formation. This polymorphism results in an amino acid change that decreases the rate of NMH synthesis.

References


Method

NMH is extracted from urine using solid phase extraction. The elute is analyzed using liquid chromatography/tandem mass spectrometry (LC-MS/MS) and quantified using a stable isotope labeled internal standard. (Martens-Lobenhoffer J, Neumann HJ: Determination of 1-methylhistamine and 1-methylimidazole acetic acid in human urine as a tool for the diagnosis of mastocytosis. J Chromatogr B Biomed Sci Appl 1999;721[1]:135-140)

Specimen Required: 5 ml from a 24-hour urine collection. No preservative. See “Urine Preservatives-Mayo Medical Laboratories” in Special Instructions (2002 Test Catalog) for other acceptable preservatives. Send specimen refrigerated in a plastic, 13-mL urine tube.

NOTE: 1. 24-hour collections are preferred, but random specimens are acceptable.
2. WHEN SUBMITTING A 24-HOUR COLLECTION, A 24-HOUR VOLUME IS REQUIRED ON REQUEST FORM FOR PROCESSING.

Reference Values: 0 - 5 years: 120 - 510 µg/g creatinine
6 - 16 years: 70 - 330 µg/g creatinine
>16 years: 30 - 200 µg/g creatinine

Analytic Time: 1 day
Days Set Up: Monday, Thursday
Fee: $108.00
CPT Code: 83789
Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC—at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in one of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

While modified guidelines for identifying such families has been introduced, there are now laboratory approaches that may also aid in establishing this diagnosis in a subset of patients with HNPCC. These approaches have arisen from studies that revealed the underlying genetic defect in many but not all families diagnosed with HNPCC, namely defective DNA mismatch repair (MMR).

Current data suggest that 50-60% of HNPCC families will have a hereditary defect in a MMR gene as the underlying genetic cause (hereditary defective MMR). Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:
• 40% associated with a mutation in hMSH2
• 40% associated with a mutation in hMLH1
• 10% associated with a mutation in hMSH6
• 10% other (unknown)
The genes involved in the remaining cases of HNPCC not involving defective MMR (approximately 40%) are, at this time, unknown.

Because patients with HNPCC may have MMR defects due to mutations in one of many genes, it is important to first identify the responsible gene prior to performing this blood test. This can be accomplished by performing #17073 “Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen”, which analyzes the patient’s tumor tissue for the presence of tumor microsatellite instability (MSI) and for an absence of protein expression (demonstrated by immunohistochemistry) for 1 of the 3 commonly affected MMR genes: hMSH2, hMLH1 or hMSH6. A stable tumor phenotype and normal protein expression suggest that the familial colon cancer is not due to defective MMR and further testing of MMR genes is not warranted. The presence of MSI and an absence of protein expression for one of the MMR genes support the presence of hereditary defective MMR. Additionally, this testing provides information on the gene that should be targeted for gene analysis (eg, hMLH1).

**MLH1 Mutation Screen #83015**

Mutation screening for hMLH1 and hMSH2 should be performed following microsatellite instability and immunohistochemistry testing on the tumor tissue of the patient. For information on this test please refer to #17073 “Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen”.

**Clinical**

Hereditary nonpolyposis colorectal cancer (HNPPC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPPC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPPC—at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in one of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

While modified guidelines for identifying such families have been introduced, there are now laboratory approaches that may also aid in establishing this diagnosis in a subset of patients with HNPPC. These approaches have arisen from studies that revealed the underlying genetic defect in many but not all families diagnosed with HNPPC, namely defective DNA mismatch repair (MMR).

Current data suggest that 50-60% of HNPPC families will have a hereditary defect in a MMR gene as the underlying genetic cause (hereditary defective MMR). Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:
• 40% associated with a mutation in hMSH2
• 40% associated with a mutation in hMLH1
• 10% associated with a mutation in hMSH6
• 10% other (unknown)
The genes involved in the remaining cases of HNPPC not involving defective MMR (approximately 40%) are, at this time, unknown.

Because patients with HNPPC may have MMR defects due to mutations in one of many genes, it is important to first identify the responsible gene prior to performing this blood test. This can be accomplished by performing #17073 “Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen”, which analyzes the patient’s tumor tissue for the presence of tumor microsatellite instability (MSI) and for an absence of protein expression (demonstrated by immunohistochemistry) for 1 of the 3 commonly affected MMR genes: hMSH2, hMLH1 or hMSH6. A stable tumor phenotype and normal protein expression suggest that the familial colon cancer is not due to defective MMR and further testing of MMR genes is not warranted. The presence of MSI and an absence of protein expression for one of the MMR genes support the presence of hereditary defective MMR. Additionally, this testing provides information on the gene that should be targeted for gene analysis (eg, hMLH1).

**Useful For**

• Determining whether absence of hMLH1 (evidence of defective mismatch repair), demonstrated by immunohistochemistry on tumor tissue, is associated with a germline mutation in the affected individual
• Characterizing the gene sequence of the mutation identified

**Interpretation**

An interpretive report will include specimen information, pedigree (when appropriate), assay information, and whether or not results are consistent with a diagnosis of HNPPC, or indicate a risk to develop HNPPC.
Cautions

- The absence of hMLH1 protein expression in the patient’s tumor tissue must be demonstrated prior to performing this test (Hereditary Nonpolyposis Colorectal Cancer HNPCC Screen).
- Data from our laboratory indicates that the direct mutation analysis using the above methods has a detection rate of approximately 90% for mutations in the hMLH1 gene. This test does not detect possible mutations in the promoter region. Thus, we predict that some individuals who have a diagnosis of HNPCC and the involvement of hMLH1 may have a mutation that is not identified by the methods described above. Accordingly, the absence of a mutation does not rule out the diagnosis of HNPCC.
- The MSI-H phenotype and the absence of protein expression for hMLH1 detected for the tumor of the patient could be the result of a somatic alteration rather than a germline mutation. In this case, testing of other affected family members may be helpful in establishing the hereditary nature of the disorder.
- In rare cases, DNA alterations of undetermined significance may be found.
- We strongly recommend that patients undergoing predictive testing receive genetic counseling both prior to testing and after results are available.

References


Method

A mutation scanning technique, conformation sensitive gel electrophoresis (CSGE), is used to analyze the hMLH1 gene for the presence of a mutation. All changes detected are then verified and characterized by DNA sequence analysis. Additionally, Southern Blot analysis is used to test for the presence of larger deletions in this gene. This assay utilizes 3 DNA probes along with 3 separate restriction enzyme digests (Eco RI, Bgl II, and Hind III). (Korkko J, Annunen S, Pihlajamaa T, et al: Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 1998 February 17;95(4):1681-1685)

Specimen Required: Specimen should include a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) with relevant clinical information and cytogenetics results, if available. Specimen must arrive within 72 hours of draw.

Draw 2 lavender-top (EDTA) tubes of whole blood (10 mL) or a yellow-top (ACD) tube(s) and send in the original VACUTAINER(S). Invert several times to mix blood. Forward unprocessed whole blood promptly at ambient temperature only.

Reference Values: An interpretive report will be issued.

NOTE: Assay performed using analyte-specific reagent. See “Analyte-Specific Reagents (ASR) - Mayo Medical Laboratories” in Special Instructions.

Analytic Time: 14 days
Days Set Up: Specimens received by Friday at 12:00 noon will be set up on the next run. This assay is run every Monday and requires 2 weeks to completion.
Fee: $439.30
CPT Code:
- 83891X1/DNA extraction
- 83894x6/Electrophoresis
- 83901x6/Each multiplex PCR
- 83903x19/Mutation scanning
- 83912x1/Interpretation and report
Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC: at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in 1 of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

While modified guidelines for identifying affected families has been introduced, new laboratory approaches may also aid in establishing this diagnosis in a subset of patients with HNPCC. These approaches have arisen from studies that revealed the underlying genetic defect in many, but not all, families diagnosed with HNPCC, namely defective DNA mismatch repair (MMR).

Current data suggest that 50-60% of HNPCC families will have a hereditary defect in a MMR gene as the underlying genetic cause (hereditary defective MMR). Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:

- Forty percent associated with a mutation in hMSH2
- Forty percent associated with a mutation in hMLH1
- Ten percent associated with a mutation in hMSH6
- Ten percent other (unknown)

The genes involved in the remaining cases of HNPCC not involving defective MMR (approximately 40%) are, at this time, unknown.

Because patients with HNPCC may have MMR defects due to mutations in 1 of many genes, it is important to first identify the responsible gene prior to performing this blood test. This can be accomplished by performing #17073 “Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen”, which analyzes the patient’s tumor tissue for the presence of tumor microsatellite instability (MSI) and for an absence of protein expression (demonstrated by immunohistochemistry) for 1 of the 3 commonly affected MMR genes: hMSH2, hMLH1, or hMSH6. A stable tumor phenotype and normal protein expression suggest that the colon cancer is not due to defective MMR and further testing of MMR genes is not warranted. The presence of MSI and an absence of protein expression for 1 of the MMR genes support the presence of hereditary defective MMR and provides information on the gene that should be targeted for gene analysis (eg, hMSH2).

**Useful For**

- Determining whether absence of hMSH2 (evidence of defective mismatch repair), demonstrated by immunohistochemistry on tumor tissue, is associated with a germline mutation in the affected individual
- Characterizing the gene sequence of the mutation identified

**Interpretation**

An interpretive report will include specimen information, pedigree (when appropriate), assay information, and whether or not results are consistent with a diagnosis of HNPCC or indicate a risk to develop HNPCC.
Cautions

- The absence of hMSH2 protein expression in the patient’s tumor tissue must be demonstrated prior to performing this test (#17073 “Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Screen”).
- Data from our laboratory indicates that the direct mutation analysis using the methods described below has a detection rate of approximately 90% for mutations in the hMSH2 gene. Additionally, this test does not detect possible mutations in the promotor region. Thus, we predict that some individuals who have a diagnosis of HNPCC and the involvement of hMSH2 may have a mutation that is not identified by the methods described above. Accordingly, the absence of a mutation does not rule out the diagnosis HNPCC.
- The MSI-H phenotype and the absence of protein expression for hMSH2 detected for the tumor of the patient could be the result of a somatic alteration rather than a germline mutation. In this case, testing of other affected family members may be helpful in establishing the hereditary nature of the disorder.
- In rare cases, DNA alterations of undetermined significance may be found.
- We strongly recommend that patients undergoing predictive testing receive genetic counseling both prior to testing and after results are available.

References


Method

A mutation scanning technique, conformation-sensitive gel electrophoresis (CSGE), is used to analyze the hMSH2 gene for the presence of a mutation. All changes detected are then verified and characterized by DNA sequence analysis. Additionally, Southern Blot analysis is used to test for the presence of larger deletions in this gene, if necessary. This assay utilizes 3 DNA probes along with 3 separate restriction enzyme digests (Eco RI, Bgl II, and Hind III). (Korkko J, Annunen S, Pihlajamaa, et al: Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 1998 February 17;95(4):1681-1685)

Specimen Required: Specimen should include a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) with relevant clinical information and cytogenetics results, if available. Specimen must arrive within 72 hours of draw.

Draw 2 lavender-top (EDTA) tube(s) of whole blood (10 mL) or a yellow-top (ACD) tube(s) and send in the original VACUTAINER(S). Invert several times to mix blood. Forward unprocessed whole blood promptly at ambient temperature only.

Reference Values: An interpretive report will be issued.

NOTE: Assay performed using analyte-specific reagent. See "Analyte-Specific Reagents (ASR) – Mayo Medical Laboratories" in Special Instructions.

Analytic Time: 14 days
Days Set Up: Specimens received by Friday at 12:00 noon will be set up on the next run. This assay is run every Monday and requires 2 weeks for completion.
Fee: $380.20
CPT Code: 83891/DNA extract-purify
83894x4/Separation by gel electrophoresis
83901x4/Each multiplex PCR
83903x16/Mutation scanning
83912/Interpretation and report
Short-chain acyl-CoA dehydrogenase (SCAD) catalyzes the first step in the mitochondrial beta-oxidation of fatty acids with a chain length of 6 to 4 carbons. SCAD deficiency is an autosomal recessive disorder with significant phenotypic variability; developmental delay and muscle hypotonia are the most consistent symptoms. Ethylmalonic aciduria (as determined by either #80619 “Organic Acids Screen, Urine” or #81249 “Acylglycines, Quantitative, Urine”) is a common, although not specific laboratory finding in affected patients.

The SCAD gene (ACADS) is located on chromosome 12q22 and consists of 10 exons. Molecular genetic studies revealed that some patients carry SCAD gene mutations that cause complete absence of SCAD activity while others carry SCAD gene variants (511C>T;625G>A), that may confer disease susceptibility only in association with other factors. The allele frequencies in the general population of the 511C>T and 625G>A gene variants are 4% and 24%, respectively.

The diagnosis of SCAD deficiency is challenging and should be based on the clinical presentation, 2 or more findings of ethylmalonic aciduria, determination of fatty acid flux in fibroblasts ( #81927 “Fatty Acid Oxidation Probe Assay, Fibroblast Culture”), and molecular genetic analysis of the SCAD gene.

Determination of the patient's genotype pertaining to only the 2 SCAD gene variants is sufficient to confirm mild deficiency of SCAD activity in vitro. However, DNA sequencing of the SCAD gene is recommended only when the fibroblast studies are suggestive of complete SCAD deficiency.

Clinical Useful For

• Investigation of possible SCAD deficiency in conjunction with the appropriate clinical presentation, demonstration of persistent ethylmalonic aciduria, and determination of fatty acid flux in fibroblasts.
• Confirmation of mild deficiency of SCAD activity

Interpretation

An interpretive report will be provided; clinical findings, family history and other laboratory data are considered.

Cautions

• This test determines the genotype related to 2 SCAD gene variants; it is not sufficient to establish a diagnosis of SCAD deficiency.
• When used as an independent assay, the high allele frequency of the 2 SCAD gene variants in the general population precludes the ability to use positive results as a reliable indicator for the presence of SCAD deficiency.
• A negative SCAD test result does not rule out the diagnosis of SCAD deficiency because other mutations are not detected by this assay.

References

Method

PCR-based assay using LightCycler technology detects the 511C>T and 625G>A mutations within the SCAD gene. This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc. DNA derived from tissue, blood, or dried blood spots is acceptable for testing. (Andresen BS, Dobrowolski SF, O'Reilly L, et al: Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. Am J Hum Genet 2001 June;68(6):1408-1418)

Specimen Required:

Specimens should include a “Molecular Genetics Congenital Disorders Request Form” (Supply T245) or a “MayoConnect Additional Test Information” form (Supply T357) with information including relevant clinical and family history information. Specimens must arrive within 96 hours of draw.

Blood

Draw blood in a lavender-top (EDTA) tube(s) or a yellow-top (ACD) tube(s), and send 3.0 mL of whole blood in the original VACUTAINER(S). Invert several times to mix blood. Forward unprocessed whole blood promptly at ambient temperature.

Reference Values:

An interpretive report will be provided

Days Set Up:

Specimens received by Friday at 12:00 noon will be set up on the next run. This assay is run every week and requires 1 week to complete.

Fee:

$194.50

CPT Code:

83912/interpretation and report
83890/molecular extraction
83898x2/single primer pair PCR
83896x2/nucleic acid probe, each
Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC— at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in 1 of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

While modified guidelines for identifying affected families has been introduced, new laboratory approaches may also aid in establishing this diagnosis in a subset of patients with HNPCC. These approaches have arisen from studies that revealed the underlying genetic defect in many, but not all, families diagnosed with HNPCC, namely defective DNA mismatch repair (MMR).

Current data suggest that 50-60% of HNPCC families will have a hereditary defect in a MMR gene as the underlying genetic cause (hereditary defective MMR). Mutations in 3 MMR genes account for the majority of inherited (germline) mutations with approximately:

- Forty percent associated with a mutation in hMSH2
- Forty percent associated with a mutation in hMLH1
- Ten percent associated with a mutation in hMSH6
- Ten percent other (unknown)

The genes involved in the remaining cases of HNPCC not involving defective MMR (approximately 40%) are, at this time, unknown.

Once a germline mutation is identified in an affected family member, predictive testing is then available for at-risk relatives. Mutation carriers face a significantly increased risk of colorectal cancer, as well as increased risk for cancers of the endometrium, kidneys, bladder, stomach, small bowel, pancreas, and ovaries.

Known mutation testing for hMLH1 and hMSH2 can be performed once a mutation has been identified in the affected family member using one of the mutation screens.

Clinical

Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is an autosomal dominant disorder that accounts for approximately 2-4% of all colon cancer. Because individual patients often lack distinctive phenotypic features, the diagnosis of HNPCC has historically been based on family history. In the early 1990s, the Amsterdam criteria were established to define the criteria used to identify patients with HNPCC— at least 3 relatives should have histologically verified colorectal cancer and 1 of them should be a first-degree relative to the other 2; familial adenomatous polyposis should be excluded; at least 2 successive generations should be affected; in 1 of the relatives, colorectal cancer should be diagnosed under 50 years of age. Subsequently, the Amsterdam criteria were recognized to be too restrictive for clinical use.

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Useful For

Predictive testing for HNPCC (hereditary defective MMR) when an hMSH2 mutation has been identified in a family member with colon cancer.

Interpretation

An interpretive report will include specimen information, pedigree, assay information, and risk for developing HNPCC.

Cautions

- The presence of an hMSH2 mutation in an affected family member must be documented prior to performing this test.
- The absence of a mutation does not rule out the diagnosis HNPCC because more than 10% of individuals affected do not have a detectable mutation.
- We strongly recommend that patients undergoing predictive testing receive genetic counseling both prior to testing and after results are available.
Test Title: MSH2 Known Mutation
#83082

References

Method
DNA Sequencing, Southern Blot Analysis

Specimen Required:
Specimen should include a “Hematopathology/Molecular Oncology Request Form” (Supply T241) or a “MayoConnect Additional Test Information Form” (Supply T357) with relevant clinical information and cytogenetics results, if available. Specimen must arrive within 72 hours of draw.

Draw 2 lavender-top (EDTA) tube(s) of whole blood (10 mL) or a yellow-top (ACD) tube(s) and send in the original VACUTAINER(S). Invert several times to mix blood. Forward unprocessed whole blood promptly at ambient temperature only.

Reference Values:
An interpretive report will be issued.
NOTE: Assay performed using analyte-specific reagent. See “Analyte-Specific Reagents (ASR) – Mayo Medical Laboratories” in Special Instructions.

Analytic Time: 14 days
Days Set Up: Specimens received by Friday at 12:00 noon will be set up on the next run. This assay is run every Monday and requires 2 weeks to completion.
Fee: $148.30
CPT Code: 83891/DNA extraction
83912/Interpretation and report