Amino Acids, Qualitative, Plasma

#83172

Clinical

Many inborn errors of metabolism affecting amino acid metabolism have been identified. Defects of either transport or catalytic activity of enzymes involved in this pathway result in the accumulation or excessive loss of 1 or more amino acids in biological fluids.

Inborn errors of amino acid metabolism can manifest themselves at any time in a person's life, but most become evident in infancy and early childhood. Affected patients may have failure to thrive, neurological symptoms, digestive problems, psychomotor retardation, and a wide spectrum of laboratory findings. If not diagnosed promptly and treated properly, these disorders can result in poor growth, mental retardation, and death.

Any interference or unusual event in the metabolism, growth, or replication of the body's cells and tissues that affect protein and amino acid metabolism will be accompanied, often dramatically, by changes in plasma and/or urinary amino acid levels.

Plasma free amino acid levels in healthy, well-nourished humans, 10-12 hours after a meal, show only slight day-to-day fluctuations and usually little variation from individual to individual unless there are large dietary differences.

Useful For

- Diagnosing inborn errors of amino acid metabolism
- Amino acid analysis may also have clinical importance in the evaluation of several acquired conditions including endocrine, liver, muscle, neoplastic and neurological disorders; nutritional disturbances; renal failure; and burns.

Interpretation

When abnormal results are detected, a detailed interpretation is given including an overview of the results and of their significance, a correlation to available clinical information, elements of differential diagnosis, recommendations for additional biochemical testing and in vitro confirmatory studies (enzyme assay, molecular analysis), name and phone number of key contacts who may provide these studies at Mayo or elsewhere, and a phone number to reach one of the laboratory directors in case the referring physician has additional questions.

Uninformative amino acid profiles are reported as normal, with the understanding that the method may not detect certain disorders characterized by minimal or intermittent amino acid elevation, and that more specialized procedures may be warranted to detect such disorders.

Cautions

- Improper handling of specimens can result in artifactual changes in the amino acid contents.
- Drugs (eg, ampicillin, drug metabolites) and other exogenous compounds are often detected by amino acid analysis. Methods based solely on chromatographic techniques do not allow a positive identification of these compounds or a conclusive interpretation of their clinical significance, if any.
Test Title: Amino Acids, Qualitative, Plasma
#83172

Reference

Method
Qualitative amino acid analysis is performed by high-pressure liquid chromatography (HPLC) using the Pico-Tag method (Bidlingmeyer BA, et al: J Chrom 1984;336:93-104). Precolumn derivatization of the amino acids with phenylisothiocyanate is followed by separation of the derivatized amino acids by reversed phase high-pressure liquid chromatography. Amino acids are detected by measurement of the absorbance of the column effluent at 254 nm.

Specimen Required: Draw blood in a green-top (heparin) tube(s) from a fasting patient. **Thrombin-activated collection tube is not acceptable.** Spin down and send 0.5 mL (pediatric: 0.25 mL) of heparinized plasma frozen in plastic vial.

Patient's age, family history, clinical presentation (asymptomatic or acute episode), diet, and drug therapy information are required on request form for processing.

Reference Values:
An interpretive report will be provided.

Analytic Time: 3 days
Days Set Up: Monday through Friday; 9:00 a.m. (not reported on Saturdays or Sundays)
CPT Code: 82128
Galactosemia Confirmation Test, Blood #84360

Profile Information

The following tests are reflexed when indicated:

<table>
<thead>
<tr>
<th>Unit Codes</th>
<th>Reporting Title</th>
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<tr>
<td>84365</td>
<td>Galactosemia Duarte Panel</td>
<td>No</td>
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<tr>
<td>84366</td>
<td>Galactosemia Gene Analysis</td>
<td>Yes</td>
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</tbody>
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Clinical

Galactosemia is an autosomal recessive inborn error of galactose metabolism. Deficiencies of 3 clinically important enzymes that catalyze the conversion of galactose to glucose and result in galactosemia have been described: deficiency of galactokinase (#8628 Galactokinase, Blood), UDP galactose-4-epimerase (GALE), and galactose-1-phosphate uridyltransferase (GALT).

The transferase GALT deficiency, referred to as classic galactosemia or G genotype, is the most commonly occurring of the 3 disorders. Typically, individuals with classic galactosemia are diagnosed via newborn screening and a lactose-restricted diet is initiated during the neonatal period. In the unfortunate circumstance where diagnosis and dietary treatment are delayed, infants usually present with gram-negative sepsis, inanition (weakness and weight loss due to severe lack of food), failure to thrive, vomiting, life-threatening liver disease, cataracts, and ultimately, mental retardation if galactose ingestion continues. Treatment with lactose-restricted diet can typically reverse the acute symptoms. Mild growth retardation, cognitive impairment, and intellectual deficit have been variably described as complications of treated galactosemia in some patients. Ovarian dysfunction is an almost unavoidable consequence of classic galactosemia, even with adherence to a strict diet. Osteoporosis secondary to decreased calcium intake can also occur.

Several disease-causing mutations are commonly encountered in classic galactosemia. The most frequently observed is the Q188R classic mutation, which has been reported to account for 54-70% of classic galactosemia alleles. The S135L mutation is the most frequently observed mutation in African Americans and accounts for approximately 50% of the mutant alleles in this population. The K285N mutation is common in those of eastern European descent and accounts for 8% of the alleles in the general European population. The L195P mutation is observed in 2.6% of classic galactosemia cases.

The Duarte N314D mutation is found in 5% of the general US population and, when paired with a classic galactosemia mutation, results in D/G mixed heterozygote. D/G mixed heterozygosity typically results in a benign phenotype, but may be treated with a low lactose-containing diet during infancy. D/G mixed heterozygotes may mimic classic galactosemia in the biochemical assays used in newborn screening (reduced enzyme activity). Thus, the relatively high frequency of the Duarte N314D mutation accounts for a significant proportion of false-positive results that occur in newborn screening programs. The silent mutation (L218L), termed the D1 Duarte variant or Los Angeles, is uncommon and associated with increased GALT enzyme activity.

A patient with a GG genotype must be on a galactose-free diet. Additionally, patients with a DG genotype must be monitored closely, and in some cases dietary treatment may be recommended.

Useful For

- Diagnosis, carrier detection, and determination of genotype of GALT deficiency, the most common cause of galactosemia
- Differentiating D/G mixed heterozygotes from classic galactosemia
- Confirming results of newborn screening programs
Interpretation

This test detects 4 of the most frequently encountered classic galactosemia alleles (Q188R, S135L, K285N, and L195P), as well as the N314D Duarte and L218L Los Angeles variants.

The laboratory provides an interpretation of the results, including GALT enzyme activity, genotype, and biochemical phenotype, if necessary. This interpretation provides an overview of the results and their significance, a correlation to available clinical information, elements of differential diagnosis, and recommendations for additional testing.

When GALT enzyme activity is in the normal range (>18.5 U/g hemoglobin), the laboratory will only test for the Los Angeles and Duarte variants, since the probability of having the G mutant allele would be unlikely. Any specimen where enzyme activity is <18.5 U/g hemoglobin will be analyzed for the presence of the 4 mutations associated with classic galactosemia, as well as the 2 variants (Duarte and Los Angeles). Cost will correlate with testing performed. See “Galactosemia Confirmation Performance Algorithm” for testing algorithm.

Galactosemia occurs in patients whose enzymes levels are extremely low.

For dietary compliance, refer to #80337 Galactose-1-Phosphate, (G-1-P), Erythrocytes.

Cautions

- Many disorders may present with symptoms similar to galactosemia. Therefore, biochemical testing is recommended to establish the diagnosis of galactosemia prior to DNA analysis.
- Not all individuals with galactosemia demonstrate the mutations included in this panel. The absence of such mutations, therefore, does not eliminate the possibility of a mutation in another region of the gene.
- This assay is not useful for monitoring dietary compliance by galactosemics, see #80337 Galactose-1-Phosphate (G-1-P), Erythrocytes. Any error in the diagnosis or in the pedigree provided to us, including false paternity, could lead to erroneous interpretations of results.
- Medical genetic consultation is available for all DNA diagnosis cases, and is particularly indicated in complex cases or when the diagnosis is atypical or uncertain.

References


Method

GALT converts uridine diphosphoglucose (UDPG) to UDP-galactose. The amount of UDPG consumed is measured by oxidizing UDPG with concomitant generation of NADPH from NADP (UDPG-dehydrogenase), which is measured at 340 nm. (Beutler E, Baluda MC: Improved method for measuring galactose-1-phosphate uridyltransferase activity of erythrocytes. Clin Chim Acta 1966 March;13(3):369-379)


A high proportion (20%) of patients with classic galactosemia have a private mutation. Since our assay does not investigate for the presence of private mutations, when GG, DG, or NG genotype is predicted by enzymatic studies and the current panel does not identify a mutation, isoelectric focusing for biochemical phenotyping will be performed.

The isoenzyme bands are visualized by applying a substrate mixture that results in a series of reactions. The final product, NADPH, is stained a blue-violet color when it reacts with phenazine methosulfate (PMS) and 3-(4-5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. (Shin YS, Niedermeier HP, Endres W, et al: Agarose gel isoelectrofocusing of UDP-galactose pyrophosphorylase and galactose-1-phosphate uridytransferase: developmental aspect of UDP-galactose pyrophosphorylase. Clin Chim Acta 1987;166:27-35)
Test Title: Galactosemia Confirmation Test, Blood #84360

Specimen Required: Draw blood in a lavender-top (EDTA) tube(s), and send 5.0 mL (pediatric: 3.0 mL) of EDTA whole blood refrigerated. **Specimen cannot be frozen.**

Reference Values:
An interpretive report will be provided.

Analytic Time: 3 days

Days Set Up: Monday through Friday; 9 a.m.

CPT Code: 82775

**Galactosemia Confirmation Performance Algorithm #84360 Galactosemia Confirmation Test, Blood**

- **Measure galactose-1-phosphate uridylyltransferase (GALT, also known as GPUT):**
  - #8333 Galactose-1-Phosphate Uridyltransferase, Blood

- **If GALT <18.5**
  - Possible classic galactosemia, galactosemia carrier, or Duarte/galactosemia mixed heterozygote. Automatically perform galactosemia gene analysis (6 mutations).
    - Q188R
    - S135L
    - K285N
    - L195P
    - Duarte: N314D
    - Duarte variant (D1 Los Angeles): L218L

- **If GALT >18.5**
  - Classic galactosemia unlikely. Automatically perform Duarte panel (2 mutations).
    - Duarte: N314D
    - Duarte variant (D1 Los Angeles): L218L

- **If no mutation detected, then isoelectric focusing (IEF) #80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes is performed to identify private mutations.**
Galactosemia Gene Analysis (6-Mutation Panel)  
#84366

Clinical

Classic galactosemia is a genetic disease caused by mutations in the galactose-1 phosphate uridyltransferase (GALT) gene. The complete or near complete deficiency of this enzyme is life threatening and affects not only the eye lens but also liver, kidney, and brain. Galactosemia is treated by removal of galactose from the diet, which allows for fast recovery from the acute symptoms and a good prognosis. Newborn screening tests, which identify potentially affected individuals, measure total galactose (galactose and galactose-1-phosphate) and/or determine the activity of the GALT enzyme on the collected dried blood specimen on filter paper.

Several disease-causing mutations are commonly encountered in classic galactosemia (G/G genotype). The most frequently observed is the Q188R classic mutation, which has been reported to account for 54-70% of classic galactosemia alleles. The S135L mutation is the most frequently observed mutation in African Americans and accounts for approximately 50% of the mutant alleles in this population. The K285N mutation is common in those of eastern European descent and accounts for 8% of the alleles in the general European population. The L195P mutation is observed in 2.6% of classic galactosemia.

The Duarte mutation (N314D) gives a GALT protein with approximately 50% of the activity of the wild-type enzyme. The Duarte variant (D) is found in 5% of the general US population and when paired with a classic galactosemia mutation results in D/G mixed heterozygote, which is probably benign but is often treated with a low-galactose-containing diet during infancy. D/G mixed heterozygotes may mimic classic galactosemia in the biochemical assays used in newborn screening. The silent mutation (L218L), termed the Los Angeles (LA) or D1 Duarte variant, is uncommon and associated with increased GALT enzyme activity, but the biochemical phenotyping (by isoelectric focusing) is identical to that of Duarte variant.

Useful For

- Confirming diagnosis of galactosemia
- Identifying galactosemia carriers in families where there is an affected individual of known genotype
- Documenting germline mutations
- Resolution of potential Duarte and Duarte/LA genotypes
- Follow-up testing for low or borderline GALT enzyme tests

Interpretation

The interpretive report includes an overview of the results and their significance, a correlation to available clinical information, elements of differential diagnosis, and recommendations for additional testing. For diagnostic purposes, results should be interpreted in the context of biochemical results.

Cautions

- Many disorders may present with symptoms similar to those present in galactosemia. Therefore, biochemical testing is recommended to establish the diagnosis of galactosemia prior to DNA analysis. This panel will be run only when the GALT enzyme level is <18.4 U/g of hemoglobin (GALT reference range=18.5-28.5 U/g of hemoglobin) or if enzyme testing cannot be performed. Not all individuals with galactosemia demonstrate the mutations included in this panel. The absence of such mutations, therefore, does not eliminate the possibility of the presence of a mutation in another region of the gene.
- Any error in the diagnosis or in the pedigree provided to us, including false paternity, could lead to erroneous interpretations of results.
- Medical genetic consultation is available for all DNA diagnosis cases and is particularly indicated in complex cases or in situations in which the diagnosis is atypical or uncertain.
Galactosemia Gene Analysis (6 mutation panel)

#84366

References


Method

In this direct mutation analysis, a polymerase chain reaction (PCR)-based assay utilizing fluorescence resonance energy transfer (FRET) probes, melt-curve analysis, and LightCycler technology are used to examine the GALT gene for the presence of the 6 mutations (see Clinical Information above). (Wittwer CT, Herrmann MG, Gundry CN, et al: Real-time multiplex PCR assays. Methods 2001 Dec;25[4]:430-432)

Specimen Required:

“Molecular Genetics - Congenital Inherited Diseases Patient Information Sheet” (Supply T521) is required for all orders. If not ordering electronically, please submit the above information sheet along with a “Molecular Genetics Congenital Disorders Request Form” (Supply T245) with the specimen.

Specimens must arrive within 96 hours of draw.

Submit 1 of the following specimens:

Blood

Draw blood in a lavender-top (EDTA) 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.

Prenatal Specimens

All prenatal specimens must be accompanied by a maternal blood specimen. Due to the complexity of prenatal testing, consultation with the laboratory is required for all prenatal testing.

Amniotic Fluid

Obtain 20 mL of amniotic fluid. Transfer specimen to 2 screw-capped, sterile centrifuge tubes. Send specimen refrigerated. Specimen cannot be frozen. A separate culture charge will be assessed under #80334 “Amniotic Fluid Culture for Genetic Testing.” Alternatively, we will accept 2 T-25 flasks of confluent cultured cells from another laboratory sent at ambient temperature.

Chorionic Villus

Obtain 20 mg of chorionic villus specimen. Send specimen refrigerated in transport media in 15-mL centrifuge tube. Specimen cannot be frozen. A separate culture charge will be assessed under #80333 “Fibroblast Culture for Genetic Testing.” Alternatively, we will accept 2 T-25 flasks of confluent cultured cells from another laboratory sent at ambient temperature.

Reference Values:

Interpretive report provided.

Analytic Time: 3 days

Days Set Up: Friday/10 am

CPT Code:

83890/Molecular diagnostics; molecular isolation or extraction
83896/x6 Nucleic acid probe, each
83898/x6 Amplification of patient nucleic acid single primer pair
83912/Interpretation and report
Galactosemia Gene Analysis: Known Mutation
#84367

Clinical

Classic galactosemia is a genetic disease caused by mutations in the galactose-1 phosphate uridylytransferase (GALT) gene. The complete or near complete deficiency of this enzyme is life-threatening and affects not only the eye lens but also liver, kidney, and brain. Galactosemia is treated by removal of galactose from the diet, which allows for fast recovery from the acute symptoms and a good prognosis. Newborn screening tests, which identify potentially affected individuals, measure total galactose (galactose and galactose-1-phosphate) and/or determine the activity of the GALT enzyme on the collected dried blood specimen on filter paper.

Several disease-causing mutations are commonly encountered in classic galactosemia. The most frequently observed is the Q188R classic mutation, which has been reported to account for 54-70% of classic galactosemia alleles. The S135L mutation is the most frequently observed mutation in African Americans and accounts for approximately 50% of the mutant alleles in this population. The K285N mutation is common in those of eastern European descent and accounts for 8% of the alleles in the general European population. The L195P mutation is observed in 2.6% of classic galactosemia.

The Duarte mutation (N314D) gives a GALT protein with approximately 50% of the activity of the wild-type enzyme. The Duarte variant is found in 5% of the general US population and when paired with a classic galactosemia mutation results in D/G mixed heterozygote, which is probably benign, but is often treated with a low-galactose-containing diet during infancy. D/G mixed heterozygotes may mimic classic galactosemia in the biochemical assays used in newborn screening. The silent mutation (L218L), termed the Los Angeles or D1 Duarte variant, is uncommon and associated with increased GALT enzyme activity, but the biochemical phenotyping (by isoelectric focusing) is identical to that of Duarte variant.

Useful For

• Confirmation of diagnosis of galactosemia in a sibling or other relative of an individual with documented GALT mutations
• Predictive testing of potential galactosemia carriers in families of individuals with documented GALT mutations

Interpretation

An interpretative report includes an overview of the results and their significance, a correlation to available clinical information, elements of differential diagnosis, and recommendations for additional testing. For diagnostic purposes, results should be interpreted in the context of biochemical results.

Cautions

• This test should be ordered only when there is a family member with a mutation previously documented by molecular testing.
• Not all individuals with galactosemia demonstrate the mutations included in this panel. The absence of such mutations, therefore, does not eliminate the possibility of the presence of a mutation in another region of the gene.
• Any error in the diagnosis or in the pedigree provided to us, including false paternity, could lead to erroneous interpretations of the results.
• Medical genetic consultation is available for all DNA diagnosis cases and is particularly indicated in complex cases or in situations in which the diagnosis is atypical or uncertain.
Galactosemia Gene Analysis: Known Mutation
#84367

Test Title: Galactosemia Gene Analysis: Known Mutation
#84367

References

Method
In this direct mutation analysis, a polymerase chain reaction (PCR)-based assay utilizing fluorescence resonance energy transfer (FRET) probes, melt-curve analysis, and LightCycler technology are used to examine the \textit{GALT} gene for the presence of the known familial mutation(s) (see Clinical Information above). (Wittwer CT, Herrmann MG, Gundry CN, et al: Real-time multiplex PCR assays. Methods 2001 Dec;25[4]:430-432)

Specimen Required:
“Molecular Genetics-Congenital Inherited Diseases Patient Information Sheet” (Supply T521) is required for all orders. If not ordering electronically, please submit the above information sheet along with a “Molecular Genetics Congenital Disorders Request Form” (Supply T245) with the specimen.

Specimens must arrive within 96 hours of draw.

Submit 1 of the following specimens:

Blood
Draw blood in a lavender-top (EDTA) 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.

Prenatal Specimens
All prenatal specimens must be accompanied by a maternal blood specimen. Due to the complexity of prenatal testing, consultation with the laboratory is required for all prenatal testing.

Amniotic Fluid
Obtain 20 mL of amniotic fluid. Transfer specimen to 2 screw-capped, sterile centrifuge tubes. Send specimen refrigerated. \textbf{Specimen cannot be frozen}. A separate culture charge will be assessed under #80334 “Amniotic Fluid Culture for Genetic Testing.” Alternatively, we will accept 2 T-25 flasks of confluent cultured cells from another laboratory sent at ambient temperature.

Chorionic Villus
Obtain 20 mg of chorionic villus specimen. Send specimen refrigerated in transport media in 15-mL centrifuge tube. \textbf{Specimen cannot be frozen}. A separate culture charge will be assessed under #80333 “Fibroblast Culture for Genetic Testing.” Alternatively, we will accept 2 T-25 flasks of confluent cultured cells from another laboratory sent at ambient temperature.

Reference Values:
An interpretive report will be issued.

Analytic Time: 3 days
Days Set Up: Friday/10 am
CPT Code:
83890/Molecular diagnostics; molecular isolation or extraction
83896/Nucleic acid probe, each
83898/Amplification of patient nucleic acid single primer pair
83912/Interpretation and report
Parvovirus B19 Antibodies, IgG & IgM, Serum
#84325

With the introduction of this test, #90380 Parvovirus (B19) IgG and IgM Antibody Panel, ELISA has been discontinued.

### Profile Information

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<tr>
<th>Unit Code</th>
<th>Reporting Title</th>
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<tbody>
<tr>
<td>81109</td>
<td>Parvovirus B19, Ab, IgG</td>
<td>No</td>
</tr>
<tr>
<td>80346</td>
<td>Parvovirus B19, Ab, IgM</td>
<td>No</td>
</tr>
</tbody>
</table>

### Clinical

Parvovirus B19 is the causative agent of fifth disease (erythema infectiosum, slapped cheek syndrome), which usually produces a mild illness characterized by an intensive erythematous maculopapular facial rash. Most outbreaks of parvovirus infection are acquired by direct contact with respiratory secretions and occur in the spring of the year. Close contact between individuals is responsible for infection in schools, daycare centers, and hospitals. The virus has also been associated with fetal damage (hydrops fetalis), aplastic crisis, and arthralgia. Infection during pregnancy risks transmission to the fetus, which may cause intrauterine death. The rate of fetal death following maternal infection ranges between 1-9%.

### Useful For

- Diagnosing recent parvovirus infection (IgM)
- Assessing past infection (eg, screening pregnant women) and immunity to parvovirus infection (IgG)

### Interpretation

Specimens with an index of <0.9 are considered negative.
Specimens with an index of >1.1 are considered positive.
Specimens with an index between 0.9 - 1.1, inclusive, are considered equivocal.

<table>
<thead>
<tr>
<th>Parvovirus B19 IgM</th>
<th>Parvovirus B19 IgG</th>
<th>Interpretation</th>
</tr>
</thead>
</table>
| Negative           | Negative           | Implies no past infection
|                    | Positive           | Implies past exposure/infection-minimal risk of B19V infection |
| Equivocal          | Positive or        | May indicate current or recent B19V infection. Retest in 1-2 weeks |
|                    | Negative           | |
| Positive           | Positive           | Implies current or recent B19V infection |
| Positive           | Negative or        | May indicate current B19V infection |
|                    | Equivocal          | Retest in 1-2 weeks |

The presence of IgM class antibodies indicates recent infection. The presence of IgG antibodies only is indicative of past exposure.

Both IgG and IgM may be present at or soon after onset of illness and reach peak titers within 30 days. Because IgG antibody may persist for years, diagnosis of acute infection is made by the detection of IgM antibodies.

The prevalence of parvovirus B19 IgG antibodies increases with age. The age-specific prevalence of antibodies to parvovirus is 2-9% of children under 5, 15-35% in children 5-18 years of age, and 30-60% in adults (19 years or older).
Test Title: Parvovirus B19 Antibodies, IgG & IgM, Serum #84325

Cautions

- Specimens taken prior to seroconversion may yield negative IgM and IgG antibody results, while specimens taken after IgM antibody levels have begun to decline may yield negative IgM antibody results. The results of a single assay or a combination of IgM and IgG enzyme immunoassays should not preclude additional testing, ie, follow-up specimens from the patient 1-4 weeks following the initial test.
- Test results of specimens from immunocompromised patients may be difficult to interpret.
- Testing should not be performed as a screening procedure for the general population.
- Specimens containing antinuclear antibodies may produce equivocal or positive test results in the IgM assay.
- Epstein-Barr virus-positive specimens may produce positive or equivocal test results in the IgM assay.

References


Method

Antibody to parvovirus B19 is detected by a sandwich enzyme immunoassay for the detection of IgG or IgM class antibodies in serum or plasma (Biotrin, Dublin, Ireland). Specific parvovirus B19 antibodies in specimens bind to antigen-coated microtiter wells. Following a wash step, peroxidase-labeled rabbit antihuman IgG is added that binds to parvovirus antibody. The antigen-antibody complex is detected by the addition of substrate, which turns blue in the presence of the enzyme peroxidase. (Anderson LJ, Tsou R, Parker RA, et al: Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;24[4]:522-526)

Specimen Required: Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down and send 0.5 mL of serum refrigerated.

Reference Values:

- IgG: <0.9
- IgM: <0.9

Analytic Time: Same day/1 day

Days Set Up: Monday through Saturday; 11:00 a.m.

CPT Code: 86747/x2
Clinical

Multiple myeloma is a hematologic neoplasm that generally originates in the bone marrow and develops from malignant plasma cells. There are 4 main categories of plasma cell proliferative disorders (PCPDs): asymptomatic myeloma, smoldering myeloma, indolent myeloma, and multiple myeloma. Asymptomatic myeloma patients have nonspecific symptoms that may be attributed to other diseases. Generalized bone pain, anemia, numbness or limb weakness, symptoms of hypercalcemia, and recurrent infections are all symptoms that may indicate myeloma. In smoldering myeloma there is a monoclonal protein spike, but it is stable. Indolent myeloma is a slowly progressing myeloma. As the myeloma progresses, the malignant plasma cells interfere with normal blood product formation in the bone marrow resulting in anemia and leukopenia. Myeloma also causes an overstimulation of osteoclasts, causing excessive breakdown of bone tissue without the normal corresponding bone formation. These bone lesions are seen in approximately 66% of myeloma patients. In advanced disease, bone loss may reach a degree where the patient suffers fractures easily.

Useful For

• Aiding in the diagnosis of new cases of multiple myeloma or other PCPDs
• Identifying prognostic markers based on the anomalies found

Interpretation

A neoplastic clone is detected when the percent of cells with an abnormality exceeds the normal reference range for any given probe.

Cautions

• This test is not approved by the FDA and is best used as an adjunct to existing clinical and pathologic information.
• This test should not be used to track the progression of disease.

Supportive Data

A total of 101 specimens were analyzed using the cytoplasmic immunoglobulin (cIg) fluorescence in situ hybridization (FISH) method. Of these, 81 had reasons for referral of any PCPD, while 20 had reasons for referral not related to PCPD and served as negative controls. The 20 normal value specimens were found to be normal by the cIg method. Of the 81 PCPD specimens, 45 had enough plasma cells for analysis (at least 25 plasma cells per hybridization site). Of the 45, 44 (98%) specimens were found to be abnormal for the probe sets used. Testing with current methodologies on these 45 specimens identified only 26 specimens (58%) as abnormal by non-cIg FISH analysis and only 10 specimens (22%) as abnormal by conventional chromosome analysis.

References

Plasma Cell Proliferative Disorder (PCPD), Fluorescence In Situ Hybridization (FISH)  
#83358

Test Title: Plasma Cell Proliferative Disorder (PCPD), Fluorescence In Situ Hybridization (FISH)  
#83358

Method

This test uses commercially available and home-brewed chromosome-specific fluorescent-labeled DNA probes for FISH. Bone marrow samples are processed to keep the cytoplasm of the leukocytes intact. At least 2 slides with 2 hybridization sites each are prepared using a cytospin centrifuge. Each probe set is hybridized to a separate hybridization site. Plasma cells are specifically detected by using immunoglobulin staining techniques with commercially available antibodies for kappa and lambda. Deletions or monosomies of chromosomes 13 and 17 are detected using FISH enumeration strategies. Translocation involving chromosomes 4 (FGFR3), 11 (CCND1) or 16 (c-MAF), and 14 (IGH) are detected by D-FISH strategies. For each probe set, 100 plasma cells (if possible) are scored and results for each abnormal probe(s) are expressed as present. (Shaughnessy J, Tian E, Sawyer J, et al: High incidence of chromosome 13 deletion in multiple myeloma detected by multiprobe interphase FISH. Blood 2000 Aug 15;96[4]:1505-1511)

Specimen Required: Please provide a reason for referral with each specimen. The laboratory will not delay or reject testing if this information is not provided, but appropriate testing and interpretation may be compromised.

Bone Marrow
Obtain 1.0-2.0 mL (pediatric: 1.0 mL) of bone marrow in a green-top (sodium heparin) tube(s). Invert several times to mix bone marrow. See “Chromosome Analysis Bone Marrow Instruction Form” in Special Instructions for processing instructions. (Clotted bone marrow is not acceptable.) Other anticoagulants are not recommended and are harmful to the viability of the cells. Label vial with patient's name and laboratory control number. Forward promptly at ambient temperature. Specimen cannot be frozen. Advise Express Mail or equivalent if not on courier service.

Note: If ordering electronically, please complete and submit a “MayoConnect Additional Test Information Form” (Supply T357 or see Special Instructions) with the specimen. If not ordering electronically, please complete and submit a “Hematopathology/Molecular Oncology/Cytogenetics Request Form” (Supply T241) with the specimen.

Reference Values: An interpretive report will be provided.
Analytic Time: 7 days
Days Set Up: Monday through Friday; Continuously
Test Classification: Mayo Clinic, Rochester, MN. It has not been cleared or approved by the U.S. Food and Drug Administration.
CPT Code: 88271/x8 DNA probe, each  
88275/x2 Interphase in situ hybridization  
88291/Interpretation and report