MAYO
Mayo Reference Services
supporting community healthcare
Communiqué
May 2005
A Mayo Reference Services Publication
EMH REFERENCE LABORATORY
Volume 30
Number 5

Feature

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Galactosemia: Current Testing Strategy and Aids for Test Selection

Galactosemia is an inborn error of carbohydrate metabolism that results from a deficiency of 1 of the 3 enzymes in the Leloir pathway: galactose-1-phosphate uridyltransferase (GALT), galactokinase (GALK), and uridine diphosphate galactose-4-epimerase (GALE). These enzymes catalyze the conversion of galactose to glucose. Individuals with galactosemia exhibit abnormally high concentrations of galactose and its metabolites in body tissues and fluids. The clinical manifestations of galactosemia are the result of toxicity from galactose exposure, and vary depending upon which enzyme is deficient. The primary treatment modality is dietary modification with restricted galactose consumption.

Confusion frequently exists regarding the appropriate tests to order for patients with known or suspected galactosemia. An understanding of the Leloir pathway, the clinical manifestations of galactosemia, treatment considerations, and the goals of testing can lead to appropriate testing for all patients.

Galactose Metabolism

Galactose is primarily metabolized through the Leloir pathway. The conversion of galactose to glucose-1-phosphate and energy begins when galactose is phosphorylated to galactose-1-phosphate by GALK. The GALT enzyme exchanges the glucose-1-phosphate moiety of uridine diphosphate (UDP)-glucose for galactose-1-phosphate, forming UDP-galactose and releasing glucose-1-phosphate. The glucose-1-phosphate released is used for either glycogen synthesis or further energy production. UDP-galactose can be converted back to UDP-glucose through a reversible reaction catalyzed by GALE or it can be incorporated into a variety of glycoproteins, glycolipids, and mucopolysaccharides. Minor pathways allow for the conversion of a small amount of galactose to galactitol and galactonic acid. The metabolism of galactose occurs primarily in the liver, although activity of each of these enzymes has been described in a variety of tissues including erythrocytes, leukocytes, ovaries and testes, kidney, brain, and cultured skin fibroblasts, chorionic villi, and amniocytes.

Galactosemia Subtypes

Galactose-1-Phosphate Uridyltransferase (GALT) Deficiency

Incidence

A deficiency of galactose-1-phosphate uridyltransferase (GALT) is the most common cause of galactosemia, and is often referred to as classic galactosemia. It has been described in all races, but is observed less commonly in Asians. Based upon reports by newborn screening programs, the frequency in the United States is approximately 1/30,000, although literature reports range from as low as 1/60,000 to as high as 1/10,000 live births.

Clinical Features

Clinically, classic galactosemia is the most severe form of galactosemia. Individuals typically appear normal at birth and exhibit clinical symptoms once lactose is introduced into the diet. Most patients present with liver disease including jaundice and hepatomegaly. Failure to thrive, vomiting, and diarrhea are also commonly observed in the neonatal period. If untreated, there is a high risk of sepsis and death.

The long-term prognosis for many patients with galactosemia may be complicated by cataracts, speech disabilities, poor growth, and learning and/or behavioral problems. Additionally, premature ovarian failure in

females and osteoporosis secondary to decreased calcium intake may occur. Early treatment can reverse the acute neonatal symptoms and may prevent or minimize long-term complications of galactosemia. However, despite the initiation of early therapy and ongoing compliance with strict dietary restrictions most patients still experience many of these symptoms.

Treatment

The clinical manifestations of classic galactosemia are thought to result from exposure to galactose or its metabolites arising from alternate pathways. As such, the goal is to minimize the accumulation of galactose and its metabolites. Exogenous sources of galactose are the greatest concern, and primary treatment for galactosemia targets dietary restriction. The main dietary source of galactose is from lactose, which is found in milk and other dairy products. Galactose is also found in tomato sauces, candies, and many medications. Lactose, a disaccharide, is hydrolyzed in the intestine by lactase to glucose and galactose. Typically, up to 40% of the caloric intake in infants is from lactose (breast milk, formula). In the adult diet, lactose represents only 3-4% of calories. The removal of lactose- and galactose-containing foods and medications from the diet is essential and must continue throughout life. Soy formulas and other milk substitutes are available; however, restrictions and substitutions can become very difficult socially for children and adults. Decreased intake of dairy products necessitates calcium supplementation. In addition, specific therapies may be necessary to treat complications such as hyperbilirubinemia or sepsis.

Biochemical and Molecular Genetics

Classic galactosemia is an autosomal recessive genetic disorder caused by mutations in the *GALT* gene. Individuals must inherit 2 deleterious copies of the gene, 1 from each parent, in order to have classic galactosemia. When an individual is homozygous for the normal allele, characterized as N, the GALT enzyme exhibits full (100%) activity. The Duarte mutation (N314D), termed D, and the galactosemia alleles, collectively designated G, confer approximately 50% and <5% of the normal enzyme, respectively. Table 1 outlines the expected relative enzyme activity by genotype; however, in practice, the absolute values for enzyme activity overlap considerably.

While over 170 disease-causing mutations have been identified as causing classic galactosemia (GG genotype), only a few are commonly encountered.

Table 1. Expected GALT Enzyme Activity by Genotype

Genotype	Relative Enzyme Activity (%)	Absolute Enzyme Activity (U/g Hgb)
NN	100	18.5-28.5
ND	75	14.0-18.4
NG or DD	50	8.1-13.9
DG	25	2.1-8.0
GG	<5	≥2.0

The most frequently observed G mutation is the Q188R, which accounts for 54% to 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 disease alleles in the general European population. The L195P mutation is observed in 2.6% of individuals with classic galactosemia.

The D mutation occurs in approximately 5% of the general population in the United States. Enzyme activity is approximately 50% of normal for DD homozygotes (similar to that of NG heterozygotes) and is sufficient such that these individuals do not have clinical or biochemical features of galactosemia. DG compound heterozygotes, often referred to as Duarte galactosemia, express significantly reduced enzyme activity, about 25% of normal, and may mimic classic galactosemia in the biochemical assays used in newborn screening and follow-up. Some physicians choose to treat Duarte galactosemia patients with a lactose-restricted diet during infancy. Specific questions regarding the treatment of a particular DG patient should be directed to a local geneticist or metabolic specialist.

Galactokinase (GALK) Deficiency

Galactokinase (GALK) deficiency is the second most common form of galactosemia, affecting approximately 1/250,000 live births. Individuals with GALK deficiency have a milder clinical presentation than seen in patients with GALT deficiency. The major clinical manifestation is bilateral, juvenile cataracts. GALK deficiency is treated with a lactose-restricted diet. Early treatment may prevent and/or reverse the formation of cataracts. The diagnosis is established by demonstrating deficient GALK enzyme activity in erythrocytes. Molecular genetic testing is currently not available on a clinical basis.

Uridine Diphosphate Galactose-4-Epimerase (GALE) Deficiency

Uridine diphosphate galactose-4-epimerase (GALE) deficiency is a very rare from of galactosemia. Features of GALE deficiency include sensorineural deafness and symptoms similar to that observed in classic galactosemia, particularly liver disease and failure to thrive. Treatment for GALE deficiency is a lactose-restricted diet. Typically patients present in the newborn period following positive newborn screen results. Testing for GALE deficiency should occur when a patient presents with clinical symptoms and/or expresses elevated levels of galactose-1-phosphate in the absence of a GALT deficiency. The diagnosis is established by demonstrating deficient GALE enzyme activity. GALE enzyme analysis is not available from Mayo Medical Laboratories (MML), but a specimen can be forwarded upon request to a clinical laboratory that performs the test. Molecular genetic testing is currently not available on a clinical basis.

Genetic Counseling

All forms of galactosemia are inherited in an autosomal recessive manner. Individuals who carry 1 copy of the gene are unaffected normal carriers, but are at risk to have a child with galactosemia. Affected individuals have 2 copies of the abnormal gene. Typically, individuals are unaware that they are carriers for galactosemia until the birth and diagnosis of a child with galactosemia. Couples with an affected child have a 25% (1 in 4) risk of recurrence in each subsequent pregnancy. Genetic counseling to discuss recurrence risks, implications for other family members, testing options, and reproductive options should be offered to all families affected by galactosemia.

Testing for Galactosemia

Patients with galactosemia are usually diagnosed following a positive newborn screen (see Newborn Screening) or based upon their clinical presentation. Confirmation of a clinical diagnosis should always include laboratory investigations. Appropriate laboratory testing is important for determining the exact cause of galactosemia. Galactose challenges, tests based upon the patient's response to galactose ingestion, are potentially dangerous and should not be used.

Newborn Screening

Screening for galactosemia is included in the newborn screening programs of all 50 states. Such programs identify potentially affected individuals and effectively reduce the morbidity and mortality rates associated with disease complications through early diagnosis and dietary restriction. The screening assays utilized vary depending upon the state program; methods usually involve the measurement of galactose and/or galactose-1-phosphate in blood spots by microbiological assays, by galactose dehydrogenase, or by measurement of GALT activity with a fluorescent spot test. Assays measuring galactose may detect both GALT and GALK defects. Screening assays that incorporate both galactose dehydrogenase and alkaline phosphatase for measurement of galactose-1-phosphate allow for the detection of patients with GALE deficiency as well. Population screening sensitivities are optimized to detect all potential cases and, as a result, false-positive results occur. Preanalytical variables such as transport temperature and humidity may increase the number of false-positives.

The accuracy of newborn screening is dependent upon the infant's lactose intake via breast milk or formula. Insufficient lactose intake may result in a false-negative screen. In addition, all infants who have received a blood transfusion prior to collection of the newborn screening specimen should have a follow-up screen performed after 90 days posttransfusion. Specific questions regarding your state newborn screening program should be addressed by personnel affiliated with the lab performing testing for your state (http://genes-r-us.uthscsa.edu/).

To reduce the risk of life-threatening complications, treatment should be initiated immediately for all infants who screen positive for galactosemia. Diagnostic testing for galactosemia must be performed in a timely manner for all screen-positive infants.

Galactose-1-Phosphate Uridyltransferase

MML's #8333 Galactose-1-Phosphate Uridyltransferase (GALT), Blood is a quantitative measurement of the GALT enzyme activity in erythrocytes. The most common explanations for decreased enzyme activity are unaffected carriers of a *GALT* mutation (eg, NG and ND), Duarte galactosemia (eg, DD and DG), or classic galactosemia (GG). GALT results are reported as a quantitative value (reference range 18.5-28.5 U/g of hemoglobin) accompanied by an interpretive report (see Table 2). Typically, analysis of the enzyme activity is not sufficient for either diagnostic or carrier testing and should be combined with molecular analysis (the preferred method) or biochemical phenotyping (an estimation of the expected genotype, such as GG, without identification of specific mutations).

Table 2. GALT Enzyme Result Interpretation

Result (U/g Hgb)	Interpretation
≥18.5	The GALT activity in this sample is normal and not consistent with classic galactosemia (GALT deficiency). Molecular genetic testing for Duarte and Los Angeles variants to follow.
14.0-18.4	The GALT activity in this sample is not consistent with classic galactosemia (GALT deficiency). This result is most consistent with heterozygosity for a mild *GALT* mutation (ie, Normal [N]/D) However, heterozygosity for a pathogenic mutation (NG) in the *GALT* gene or homozygosity for a mild *GALT* mutation (ie, DD) cannot be ruled out.
8.1-13.9	The GALT activity in this sample is reduced; however, not consistent with classic galactosemia (GALT deficiency). Partial enzyme deficiency can be explained by a variety of genotypes.
2.1-8.0	The GALT activity in this sample is most consistent with the Duarte variant galactosemia (DG). However, heterozygosity for a classic *GALT* mutation (NG) is also possible. We recommend continuing a galactose-free diet until the diagnosis can be further clarified.
≤2.0	The GALT activity in this sample is most consistent with classic galactosemia (GALT deficiency). However, Duarte variant galactosemia (DG) cannot be ruled out. Treatment with galactose-restricted diet should be initiated immediately.

Measurement of the GALT enzyme activity is suggestive of the biochemical phenotype; however, various genotypes that result in similar enzymatic results cause ambiguity. For example, the Los Angeles mutation, termed LA, confers greater than normal activity and when paired with an N allele, elevated enzyme activities are observed. In phenotypically normal individuals, the LA mutation can mask a G allele (LA increases activity; G allele decreases activity), but the enzyme activity is likely to be similar to that of an individual with an ND genotype (lower than normal). For these reasons, a stepwise algorithm beginning with quantitative enzyme analysis followed by molecular testing is the recommended approach for diagnostic or carrier testing for galactosemia due to GALT deficiency.

Molecular Analysis

Molecular genetic analysis of the *GALT* gene is useful for confirming the diagnosis of galactosemia. Utilizing a polymerase chain reaction (PCR)-based assay, #84366 Galactosemia Gene Analysis Panel (6 Mutations) detects the presence of 6 alterations in the *GALT* gene. Included in this panel are 4 of the most frequently observed classic galactosemia mutations (Q188R, S135L, K285N, and L195P) and the Duarte (N314D) and Los Angeles (L218L) variants. GALT enzyme assay is recommended to establish the diagnosis of galactosemia prior to DNA analysis. Testing for the 4 most common G mutations will identify 70% of classic galactosemia mutations. The absence of such mutations does not rule out classic galactosemia or eliminate the possibility of a mutation in another region of the gene. The molecular panel also is useful in distinguishing individuals with low GALT activity who require lifelong treatment (GG patients) from those for whom treatment may be optional and of limited duration (DG patients).

When the specific mutations are identified in an affected individual, #84367 Galactosemia Gene Analysis: Known Mutation is useful in identifying galactosemia carriers in related family members. Similarly, this test can be used for prenatal diagnosis for at-risk pregnancies. Any error in the diagnosis of the proband or in the pedigree provided, including false-paternity, could lead to erroneous interpretations of results. As such, this test should only be ordered when the mutations in the affected family member are well documented.

Results of the molecular testing are provided in an interpretive report that includes an overview of the results and their significance, a correlation to available clinical information and results of prior biochemical genetic analyses, elements of differential diagnosis, and recommendations for additional testing if necessary. For diagnostic purposes, results should be interpreted in the context of biochemical results. Given this, the #84360 Galactosemia Confirmation Test, Blood is the most appropriate test to order when beginning the workup of a patient suspected to have galactosemia. See algorithm, Figure on page 5.

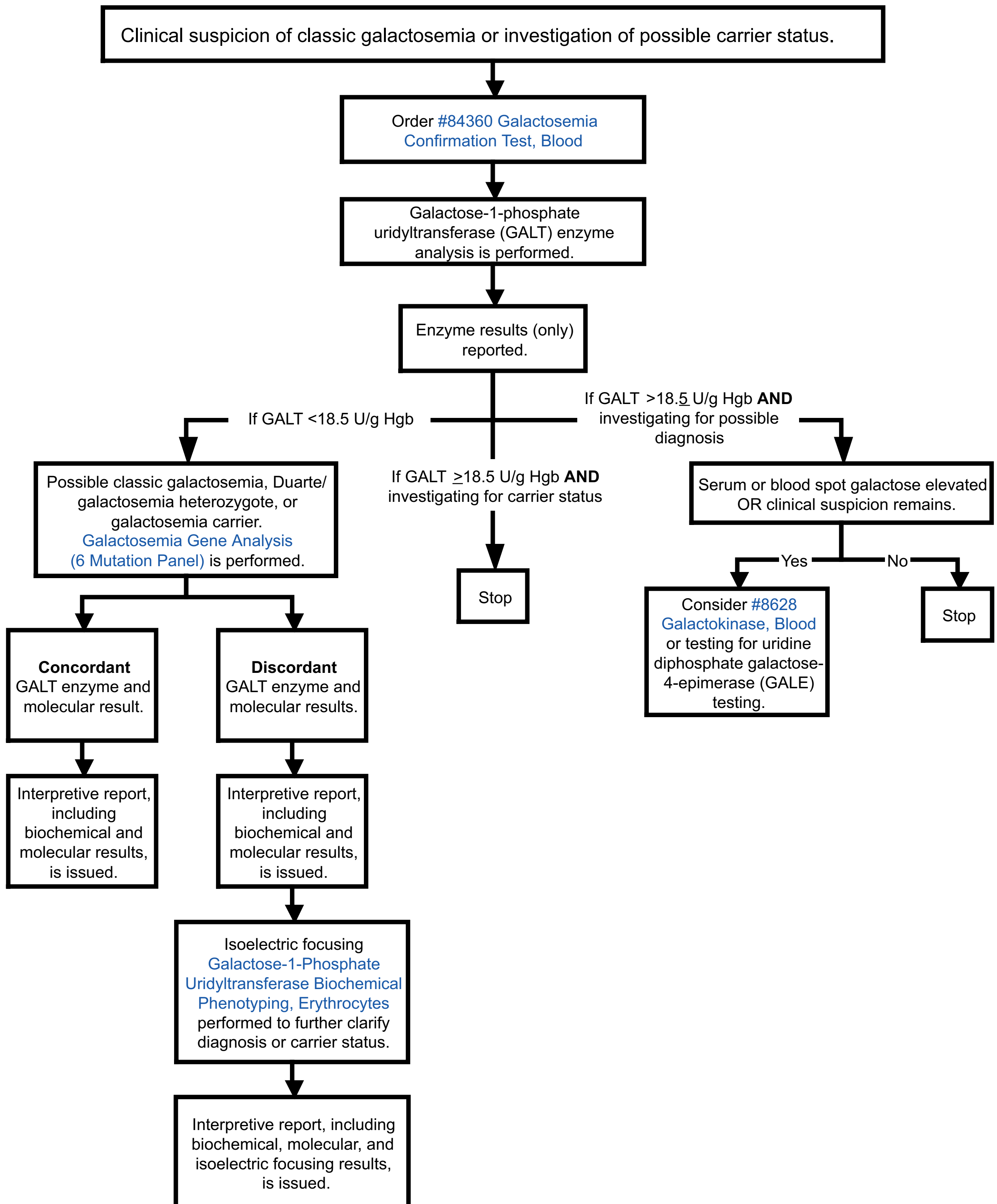

Figure. Galactosemia Confirmation Algorithm.

Galactosemia Confirmation Test
MML offers a reflex test, #84360 Galactosemia Confirmation Test, Blood, for galactosemia diagnosis and carrier detection. When the specimen arrives in the lab, an aliquot is reserved for molecular analysis and the remaining specimen is used for biochemical analyses. The galactosemia confirmation test begins with GALT quantitative enzyme analysis (#8333 Galactose-1-Phosphate Uridyltransferase (GALT), Blood). If the GALT enzyme activity is <18.5 U/g of hemoglobin the specimen is reflexed to molecular testing for a 6-mutation panel (#84366 Galactosemia Gene Analysis Panel [6 Mutations]). In these cases, the patient may have classic galactosemia or be a carrier. When the enzyme activity is within the reference range (≥18.5 U/g of hemoglobin), individuals do not have galactosemia and the probability of having a G mutation is very low.

Molecular analysis will not detect all disease-causing G mutations in the GALT gene. When a GG, DG, or NG genotype is predicted by enzymatic studies and the current molecular panel does not identify a mutation, isoelectric focusing for biochemical phenotyping (#80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes) may be necessary to resolve the results. It is estimated that in approximately 10% of cases, biochemical phenotyping may be of value. The decision to reflex to biochemical phenotyping is made on a case-by-case basis. Clients are contacted if the laboratory determines phenotype testing is appropriate.

Although #84360 Galactosemia Confirmation Test, Blood is ordered as a single test code, the results of the GALT enzyme activity and molecular analysis are reported individually. This allows for timely communication of results that may impact treatment decisions and facilitate discussions with the patient's family. Likewise, when performed, the results of biochemical phenotyping are reported separately.

Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping
MML's #80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes is useful for determining the biochemical phenotype (an estimation of the genotype) of an individual. The biochemical phenotype should be identified when a GG, DG, or NG genotype is predicted by enzymatic studies and the current molecular panel does not identify a mutation. Results are provided in the form of an interpretive comment.

Biochemical phenotyping utilizes isoelectric focusing to separate the protein on a gel. The banding pattern, when used in conjunction with a quantitative GALT result, can be used to predict the GALT biochemical phenotype of an individual. For example, while both DD and NG individuals exhibit approximately 50% of normal GALT activity, a different banding pattern is observed upon isoelectric focusing. While this test allows for an estimation of the genotype (eg, NG or GG), one cannot predict specific mutations (eg, Q188R) present in an individual because the protein is being analyzed, not the DNA. Given that the GALT enzyme analysis is utilized to aid in the interpretation of the banding pattern, if the GALT enzyme assay has not been performed previously through MML, it must be ordered at an additional charge.

Unfortunately, banding patterns are not always distinct, which may result in ambiguous results. Biochemical phenotyping is most effective when the parental phenotypes are also established. Testing and result interpretation are further complicated by the LA mutation, which while relatively uncommon and associated with an increased GALT enzyme activity, exhibits a biochemical phenotyping pattern identical to the Duarte allele. Biochemical phenotyping is technically difficult and with the advancements in molecular genetic testing, it is only recommended when the results of enzyme and molecular studies require further resolution.

To optimize testing, MML's #84360 Galactosemia Confirmation Test, Blood includes the quantitative GALT level and reflexes to molecular testing. Subsequent biochemical phenotyping is only performed in cases where further clarification is necessary. Some physicians and state newborn screening programs are opting to workup patients suspected to have galactosemia by performing both GALT enzyme activity analysis and isoelectric focusing. Although not the preferred approach, these requests will be accommodated and the Biochemical Genetics Laboratory will make recommendations regarding molecular analysis as appropriate.

Galactokinase
Diagnosis of the second most common cause of galactosemia, GALK deficiency, is accomplished via #8268 Galactokinase, Blood. Results are reported as a quantitative value (reference range in mU/g hemoglobin: <2 years: 20.1-79.8; ≥2 years: 12.1-39.7) accompanied by an interpretive report. The activity of GALK in erythrocytes is highest in newborns and decreases with age. Only results below the normal range are clinically significant; elevated values have no known

clinical significance. Results of this test are not reliable for detecting carriers.

Testing for GALK deficiency should be performed when there is a suspicion of galactosemia and the GALT deficiency has been ruled out either based upon the patient's clinical presentation or prior laboratory studies. Specimens sent for GALT analysis may be used for GALK testing if the original specimen was received in the laboratory within 48 hours.

Special Circumstances - Transfusions
It is important to notify the laboratory if a patient who is being investigated for galactosemia has received a transfusion prior to specimen collection. The results of any testing performed in erythrocytes, including analysis of the enzymes, biochemical phenotyping, or galactose-1-phosphate, are invalid following a transfusion. The donor cells may mask the patient's true results, impacting the interpretation of test results. Testing may need to be repeated after or deferred for 90 days in these cases. Biochemical and molecular studies on both parents can be helpful in making a presumptive diagnosis. Testing recommendations for specific cases will be made by Mayo staff upon request.

Additional Screening Tests
Other screening tests for galactosemia are available. These are only appropriate in certain situations and should be utilized on a limited basis.

Galactose, Plasma
Elevated plasma galactose values are found in individuals with galactosemia. #83638 Galactose, Quantitative, Plasma is a screening test that may be useful when there is a suspicion of galactosemia in individuals who have normal GALT enzyme activity or who have received a blood transfusion. Table 3 illustrates which metabolites are elevated in the various forms of galactosemia when an individual with galactosemia is on a regular, lactose-containing diet.

Table 3. Metabolite Elevations by Type of Galactosemia

Deficiency	Galactose (plasma) #83638	Galactose 1 phosphate (erythrocytes) #80337
GALK	Elevated	Normal
GALT	Elevated	Elevated
GALE	Normal – Elevated	Elevated

Galactose, Urine
Urine galactose (#8765 Galactose, Quantitative, Urine) has been used as a confirmatory test for the presence of reducing substances in the urine (eg, Benedict's test, Clinitest™). However, galactosuria may be seen in a variety of other disorders. Other tests previously discussed are recommended for the workup of patients suspected to have galactosemia.

Monitoring for Dietary Compliance

Galactose-1-Phosphate
Galactose-1-phosphate accumulates in the erythrocytes of patients with galactosemia due to a GALT or GALE deficiency. The quantitative measurement of galactose-1-phosphate, #80337 Galactose-1-Phosphate (G-1-P), Erythrocytes, is useful for monitoring compliance with dietary therapy for classic galactosemia (GG) patients, Duarte galactosemia (DG) patients, or patients with GALE deficiency. Galactose-1-phosphate is thought to be the causative factor for development of liver disease in these patients. During the initial workup of the patient, this test also can indicate the relative condition of the patient and the need for immediate intervention.

In general, patients with GALT or GALE galactosemia should maintain galactose-1-phosphate levels as low as possible. Most patients have their galactose-1-phosphate levels monitored on a regular basis. Galactose-1-phosphate results are reported as a quantitative value and reference ranges are provided for normal individuals, galactosemia patients on a restricted diet, and galactosemia patients who are not in compliance with dietary therapy (Table 4). Various laboratories may utilize different methods for analysis, units of measure, or reference ranges. The Biochemical Genetics Laboratory at Mayo defined the reference ranges based upon published values in the literature. The most conservative galactose-1-phosphate value ranges for monitoring dietary compliance are used. If results are obtained from another laboratory, and the clinician wants to compare values over time, the results must be converted such that the units of measure are consistent and the values are compared to the same reference range. If such issues are not taken into consideration, dietary modifications or other clinical decisions may be made based upon incongruous data.

Specimens collected for galactose-1-phosphate analysis must be washed per the MML protocol and shipped immediately. Specimen handling is critical as galactose-1-phosphate levels may fall up to 50% within 4 hours at room temperature. Stability studies have shown that

Table 4. Reference Ranges for Galactose-1-Phosphate

Galactose-1-phosphate (µg /g Hgb)	Interpretation
5-49	Nongalactosemic
50-125	Galactosemic on galactose-restricted diet
>125	Galactosemic on unrestricted diet

galactose-1-phosphate is stable in washed, packed erythrocytes at –20°C for 9 days. A specimen collected for galactose-1-phosphate analysis may be used for the GALT and biochemical phenotyping assays. However, the galactose-1-phosphate specimen may not be used for molecular genetic testing as there is no DNA in the washed, packed erythrocytes. Due to the limited stability of galactose-1-phosphate, whole blood specimens received for #84360 Galactosemia Confirmation Test, Blood; #8333 Galactose-1-Phosphate Uridyltransferase (GALT), Blood; or #80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes cannot be utilized for galactose-1-phosphate studies.

Conclusion

Laboratory identification of patients with galactosemia can be complicated. In follow-up to abnormal newborn screening results or a clinical suspicion of galactosemia, testing for the specific enzymatic defects should be performed in the order of probability (GALT, GALK, and GALE). Recommended for initial testing, MML's #84360 Galactosemia Confirmation Test, Blood includes GALT enzyme analysis with automatic reflex to molecular genetic testing, which simplifies test selection.

Consultation with a laboratory director or genetic counselor in the Biochemical Genetics or Molecular Genetics Laboratories is available for clinicians and laboratory personnel to discuss testing strategies and for assistance with result interpretation. These individuals can be reached by contacting Mayo Lab Inquiry at 1-800-533-1710.

Test Updates

Testosterone Method and Reference Changes
A method change for #8533 Testosterone, Total, Serum has resulted in changes to the reference values for this test and the following profiles:
#8508 Testosterone, Total and Free, Serum
#80065 Testosterone, Total and Bioavailable, Serum
#83686 Testosterone, Total, Bioavailable, and Free, Serum

New Method
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) for total testosterone.
Previous Method
Chemiluminometric immunoassay

New Reference Values (previous reference values omitted)

Age-adjusted ranges		Reference Range (ng/dL)
Males:	0-5 months	75-400 ng/dL
	6 months-9 years	<7-20 ng/dL
	10-11 years	<7-130 ng/dL
	12-13 years	<7-800 ng/dL
	14 years	<7-1,200 ng/dL
	15-16 years	100-1,200 ng/dL
	17-18 years	300-1,200 ng/dL
	≥19 years	240-950 ng/dL
Females:	0-5 months	20-80 ng/dL
	6 months-9 years	<7-20 ng/dL
	10-11 years	<7-44 ng/dL
	12-16 years	<7-75 ng/dL
	17-18 years	20-75 ng/dL
	≥19 years	8-60 ng/dL

Tanner Stages*		Reference Range (ng/dL)
Males:	I (prepubertal)	<7-20 ng/dL
	II	8-66 ng/dL
	III	26-800 ng/dL
	IV	85-1200 ng/dL
	V (young adult)	300-950 ng/dL
Females:	I (prepubertal)	<7-20 ng/dL
	II	<7-47 ng/dL
	III	17-75 ng/dL
	IV	20-75 ng/dL
	V (young adult)	12-60 ng/dL

*Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (±2) years and for girls at a median age of 10.5 (±2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African American girls. For boys there is no definite proven relationship between puberty onset and body weight or ethnic origin. Progressions through Tanner stages are variable. Tanner stage V (adult) should be reached by age 18.

Beta-2 Transferrin Method Change
Beta-2 transferrin, also called cerebrospinal fluid (CSF)-specific transferrin and tau protein, is a CSF-specific variant of transferrin and is used as an endogenous marker of CSF leakage. Prompt diagnosis and localization of CSF leakage facilitates appropriate treatment decisions and decreases the risk of meningitis. While traditional chemical analyses are unreliable, and radiographic studies involving injections may increase the risk to the patient, #80351 Beta-2 Transferrin: Detection of Spinal Fluid in other body fluids provides a minimally invasive method of detection.

The method for this assay has been changed from electrophoresis/immunofixation/silver stain to electrophoresis/immunofixation/enzyme-immunodetection. This change significantly increases the sensitivity of the assay. The reference value remains "negative, no beta-2 transferrin (spinal fluid) detected."

Ganglioside Antibody Panel Test Changes
As a result of computer billing requirements, **#80904 Ganglioside Antibody Panel, Serum** has been assigned a new unit code: #83189 Ganglioside Antibody Panel, Serum. In addition to the unit code change, there has been a change in the required specimen volume. The specimen has been reduced from 4.0 mL of serum to **1.0 mL of serum** (sent frozen in a plastic vial). No other aspect of the test has been affected by these changes.

FISH for CLL Locus Anomalies Expanded
Testing for CLL, #83089 Locus and Centromere Anomalies for Chronic Lymphocytic Leukemia (CLL), Fluorescence In Situ Hybridization (FISH), uses fluorescent-labeled DNA probes to detect common chromosome abnormalities in nonproliferating nuclei (interphase cells) in CLL. The loci tested include 6cen (D6Z1), 6q23 (c-MYB), 11cen (D11Z1), 11q13 (CCND1), 11q23 (ATM), 12cen (D12Z3), 12q15 (MDM2), 13q14 (D13S319), 13qter (D13S327), 14q32 (IgH), 17cen (D17Z1), and 17p13.1 (p53). A total of 200 interphase nuclei are scored for each probe.

To improve identification of the specific abnormality, when the initial probe results indicate an abnormality in the IgH loci (14q32) other than fusion with CCND1 (11q13), additional probes are now used to detect fusion of IgH with either BCL2 (18q21) or BCL3 (19q13.3).

Chimerism-Recipient Engraftment Method Change
The method for #84427 Chimerism-Recipient Engraftment (Post) was changed to the AmpF/STR®Profiler Plus™ ID PCR Amplification Kit. The new method provides improved accuracy and precision.

Abstracts of Interest

Chronic Myeloid Leukemia: Current Application of Cytogenetics and Molecular Testing for Diagnosis and Treatment

Ayalew Tefferi, MD; Gordon W. Dewald, PhD; Mark L. Litzow, MD; Jorge Cortes, MD; Michael J. Mauro, MD; Moshe Talpaz, MD; Hagop M. Kantarjian, MD

Chronic myeloid leukemia provides an illustrative disease model for both molecular pathogenesis of cancer and rational drug therapy. Chronic myeloid leukemia is a clonal stem cell disease caused by an acquired somatic mutation that fuses, through chromosomal translocation, the *abl* and *bcr* genes on chromosomes 9 and 22, respectively. The *bcr/abl* gene product is an oncogenic protein that localizes to the cytoskeleton and displays an up-regulated tyrosine kinase activity that leads to the recruitment of downstream effectors of cell proliferation and cell survival and consequently cell transformation. Such molecular information on pathogenesis has facilitated accurate diagnosis, the development of pathogenesis-targeted drug therapy, and most recently the application of molecular techniques for monitoring minimal residual disease after successful therapy. These issues are discussed within the context of clinical practice.

Mayo Clinic Proceedings 2005;80(3):390-402

The complete article is available online at URL: http://www.mayo.edu/proceedings/.

2005 *Education* **Calendar**

Interactive Satellite Programs . . .

Drug Cautions in the Elderly
May 10, 2005
Presenter: *David G. Bell, MD*
Moderator: *Robert M. Kisabeth, MD*

Congestive Heart Failure
June 1, 2005
Presenter: *Allan S. Jaffe, MD*
Moderator: *Robert M. Kisabeth, MD*

Laboratory and Clinical Collaboration in the Diagnosis and Management of Thyroid Disease
June 14, 2005
Presenter: *Bryan McIver, MBChB, PhD*
Moderator: *Robert M. Kisabeth, MD*

Alzheimer's: An Update on Treatment and Research
September 6, 2005
Presenter: *Ronald C. Petersen, MD, PhD*
Moderator: *Robert M. Kisabeth, MD*

Genomics & Proteomics – An Update
November 1, 2005
Presenter: *David B. Schowalter, MD, PhD*
Moderator: *Robert M. Kisabeth, MD*

What's New in Hereditary Hemochromatosis
December 13, 2005
Presenter: *David J. Brandhagen, MD*
Moderator: *Robert M. Kisabeth, MD*

Upcoming Education Conferences . . .

12th International Surgical Pathology Symposium
May 3-6, 2005
Sofitel Victoria Hotel • Warsaw, Poland

Integration Through Community Laboratory Insourcing
May 18-20, 2005
Sofitel Philadelphia • Philadelphia, Pennsylvania

4th Biennial Symposium – Pulmonary Pathology Society
June 15-17, 2005
L'Imperial Palace • Annecy, France

Coagulation Testing Quality: Lessons and Issues from Quality Assessment, Standardization and Improvement Programs & Studies
June 15-17, 2005
The Kahler Grand Hotel • Rochester, Minnesota

How the Practice of Medicine Informs Technology
July 23, 2005
Rosen Centre Hotel • Orlando, Florida

Quality Phlebotomy: Back to the Basics
September 27, 2005
Airport Marriott • Los Angeles, California

Practical Surgical Pathology
September 29-October 1, 2005
Mayo Clinic, Siebens Building • Rochester, Minnesota

Practical Spirometry
November 17-18, 2005
Mayo Clinic, Siebens Building • Rochester, Minnesota

Real-Time PCR for the Clinical Laboratory
November 17-18, 2005
Mayo Clinic, Siebens Building • Rochester, Minnesota

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3050 Superior Drive NW
Rochester, Minnesota 55901
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MC2831-0505

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