Cytochrome P450 3A4 (CYP3A4) is a member of the family of cytochrome P450 enzymes, which are involved in drug metabolism. Different variants (polymorphisms) of cytochrome P450 enzymes exist, and individuals who inherit a given variant may metabolize some drugs differently than those individuals who inherit other variants.

Various modes of phenotyping CYP3A4 have been explored, notably the administering drugs that are metabolized by CYP3A4 (eg, erythromycin or intravenous midazolam) as probe drugs, followed by assessment of drug clearance. While the safety profile of selected probe drugs is very good and the risk to the patient is minimal, such testing is considered invasive and requires advance preparation.

Cortisol (compound F) is metabolized to 6-beta-hydroxycortisol by CYP 3A4. The urinary excretion ratio of 6-beta-hydroxycortisol/cortisol has been extensively used as a noninvasive index for phenotyping hepatic CYP3A4 activity. Historically, the principal reason that steroid hydroxylation has not been the method of choice is the lack of analytical specificity in antibody-based or chromatographic techniques. The introduction of LC-MS/MS methods overcomes these concerns.

**Useful For**

Phenotyping CYP3A4 activity

**Interpretation**

Population averages are useful for classifying responses to a drug or drug combination.

Changes of significantly more than 30% within an individual may reflect either induction of the enzyme or inhibition due to a coadministered drug.

**Cautions**

- Reference ranges are laboratory specific, due to variability in analytical specificity.
- Certain single nucleotide polymorphisms (SNPs) give rise to reduced enzymatic activity, such as *4, but there is considerable overlap with the wild-type population.
- Predictive power of the results, in relation to metabolism of any particular drug, must be interpreted with caution due to the overlap of populations.
- The ratio is extremely variable following liver transplantation, and use of this test in that population must be considered speculative.

## Method

Urine specimens, controls, and standards containing the mixture of internal standard (deuterium-labeled cortisol) are first extracted with 3.0 mL of 85% ethyl acetate/15% isopropyl alcohol solution and then with 1.0 mL each 0.1 M NaOH and 0.1 M acetic acid. Following extraction, the samples are concentrated and injected into the LC-MS/MS system. The atmospheric electrospray ionization (ESI) source is used. Multiple reaction monitoring (MRM) is used to detect ion pairs at m/z 379/239 (6-beta OHF), 363/121 (F), and 367/121 (d[4]-F).


### Specimen Required:
Submit only 1 of the following specimens:

#### Urine, 24-Hour Collection
5.0 mL from a 24-hour collection. No preservative. See “Urine Preservatives” in Special Instructions for multiple collections. Send specimen frozen in a plastic, 13-mL urine tube.

**Note:**
1. Indicate 24-hour urine on request form.
2. Label specimen appropriately (24-hour urine).

#### Urine Preservative Collection Options
**Note:** The addition of preservative or application of temperature controls **must occur at the start** of the collection.

- Ambient: Yes
- Refrigerate: Yes
- Frozen: Preferred
- 6N HCl: No
- 50% Acetic Acid: Yes
- Na(2)CO(3): No
- Toluene: No
- 6N HNO(3): No
- Boric: Yes
- Thymol: No

#### Urine, Random Collection
5.0 mL from a random urine collection. Send specimen frozen in a plastic, 13-mL urine tube.

**Note:**
1. Indicate random urine on request form.
2. Label specimen appropriately (random urine).

**Reference Values:**
6-Beta-hydroxycortisol/cortisol ratio
- Mean = 6.0
- Standard deviation = 4.12
- 95% range: 2.1-14.1

**Analytic Time:**
2 days

**Days Set Up:**
Tuesday; 10 am

**CPT Code:**
82530
Endoscopic retrograde cholangiopancreatography (ERCP) is used to examine patients with biliary tract obstruction/stricture for possible malignancy. Biopsies and cytologic specimens are obtained at the time of ERCP. Cytologic analysis complements biopsy by sometimes detecting malignancy in patients with a negative biopsy. Nonetheless, a number of studies suggest that the overall sensitivity of bile duct brushing/bile aspirate cytology is quite low.

Two relatively newer ancillary techniques, digital imaging analysis (DIA) and fluorescence in situ hybridization (FISH), have both demonstrated superior sensitivities in detecting biliary tract malignancy when compared to routine cytology. In DIA, a computer is attached to a microscope and bright field nuclear images are digitalized for quantitation. The captured nuclear images represent a series of picture elements, or pixels, the optical density of which is compared to that of a standard specimen with known DNA content in picograms (pg). Plotting pg of DNA versus number of nuclei generates a histogram and allows for a DNA ploidy interpretation, which helps distinguish benign from malignant strictures of the biliary tract.

FISH is a technique that utilizes fluorescently labeled DNA probes to examine cells for chromosomal alterations. FISH can be used to detect cells with chromosomal changes (e.g., aneuploidy) that are indicative of malignancy. The FISH for biliary tract malignancy assay utilizes a multicolor, multitarget probe mixture to detect malignant cells in biliary brush and bile aspirate specimens.

The combined use of DIA and FISH increases the detection of biliary tract malignancy, independent of the cytology results (see Supportive Data).

**Useful For**

Detecting malignancy in bile duct, when used in conjunction with cytology

**Interpretation**

A significant population of cells with chromosomal gains and/or aneuploid DNA content indicates that the patient has a primary or metastatic biliary tract malignancy.

**Cautions**

Positive DIA/FISH results do not identify location or type of malignancy; cytology and biopsy may help clarify such situations.
Supportive Data

Bile brushing specimens were collected from June 2000 to October 2001 from patients with biliary strictures without a discrete mass. A portion of the specimen was prepared by the Thin Prep technique for cytologic evaluation, and the remaining specimen was split for DIA and FISH analysis. Evidence of malignancy on follow-up was based on surgical pathologic or clinical/radiographic evidence of malignancy after 1-year minimum follow-up. P values for the differences in the sensitivity and specificity of each test combination were determined with the z-test. Specimens were from 127 patients (81 men and 46 women with mean age 56.1 years; range 18.9 to 87 years). Sixty-nine patients (54%) had a diagnosis of primary sclerosing cholangitis.

Combining cytology, DIA and FISH increased the sensitivity of identifying extrahepatic bile duct malignancies when compared with cytology results alone or cytology in combination with DIA or FISH, suggesting the combination of tests may be useful in the triage and management of patients with biliary strictures.

An additional study assessed the value of FISH and DIA for the diagnosis of biliary tract malignancy. The results of this study, using univariate logistic regression analysis, suggest that FISH and DIA add significant ($P<0.01$) independent power in models predicting which patients have cancer when compared to the diagnosis made by routine cytology. Therefore, the combination of DIA and FISH will significantly increase the detection of biliary tract malignancy independent of the cytology result.

References


Method

Standard brush cytology sampling is performed on patients undergoing ERCP for suspicious biliary tract strictures. Brushes are placed in ThinPrep vials containing PreservCyt or CytoLyt solution. The sample is sent in a single vial with or without the brush. If brush is present, it is removed and cells are collected from it by scraping them into a single vial containing 20 mL of PreservCyt solution. Two aliquots are prepared and used for each portion of the test.

A ThinPrep slide is made for Feulgen staining, and DNA content of the individual nuclei is measured by lab technologists using the CAS 200 Image Analyzer. A minimum of 50 nuclei are collected and a DNA histogram is generated for DNA ploidy interpretation by a pathologist. The DNA index is determined from the modal (most common) value of DNA content in the histogram’s dominant peak. Histograms are classified as either diploid, near-diploid, tetraploid, or aneuploid.

Biliary cells are harvested, fixed, and placed on a slide. Fluorescently labeled DNA probes to the centromeres of chromosomes 3, 7, and 17, and to the 9p21 locus are hybridized to the cells on the slide (Vysis UroVysion, Abbott Laboratories, Waukegan, IL). The slide is then washed and stained with DAPI (a nuclear counterstain).
Fluorescence microscopy with unique band filters is then used to scan the slide for atypical cells (e.g., cells with nuclear enlargement or irregularity). These cells are assessed for gains of chromosomes 3, 7, and 17. If the number of cells with chromosomal gains (polysomy or trisomy) observed on scanning is sufficient to consider the test result positive, the percentage of biliary cells with polysomy or trisomy is determined. (Rumalla A, Baron TH, Leontovich O, et al: Improved diagnostic yield of endoscopic biliary brush cytology by digital image analysis. Mayo Clin Proc 2001 January;76:29-33; Baron TH, Harewood GC, Rumalla A, et al: A prospective comparison of digital image analysis and routine cytology for the identification of malignancy in biliary tract strictures. J Gastroenterol Hepatol, In press)

**Specimen Required:**
Submit bile duct brushing and/or bile aspirate, hepatobiliary brushing and/or hepatobiliary aspirate each in a separate ThinPrep vial containing PreservCyt or CytoLyt solution (Supply T536).

**A two-thirds aliquot of the original specimen is required** for DIA and FISH testing. Use of residual specimen from original processing may compromise the sensitivity of the tests.

**Note:** If ordering electronically, no form is required with the specimen. If not ordering electronically, please complete and submit a “Pathology/Cytology In Situ Request Form” (Supply T246) with the specimen.

**Reference Values:**
An interpretive report will be provided.

**Analytic Time:**
7 days

**Days Set Up:**
Monday through Friday

**CPT Code:**
88358/Biliary Tract Malignancy, DIA
88368X4 Biliary Tract Malignancy, FISH

**Test Classification:**
This test was developed and its performance characteristics determined by Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN. It has not been cleared or approved by the U.S. Food and Drug Administration. Results should be interpreted in conjunction with clinical findings.
Polyomaviruses are small (45 nm, 5,000 bp) DNA-containing viruses and include 3 closely related strains of clinical significance, SV40, JCV, and BKV. SV40 naturally infects rhesus monkeys but can infect humans; BKV and JCV cause productive infection only in humans. There is some speculation that SV40 may be involved in rare instances. Acquisition of antibody to JCV and BKV begins in infancy. The prevalence of antibody to JCV is 10% in children 5 years of age and rises to 76% by late adult life. Serological evidence of infection by BKV is present in 37% of individuals by 5 years of age and over 80% by adolescence.

Human symptomatic infections with polyomaviruses usually occur in immunocompromised patients. JCV is the causative agent of progressive multifocal leukoencephalopathy (PML) a central nervous system disease. Laboratory diagnosis is obtained by detecting viral DNA in cerebrospinal fluid (CSF) specimens by PCR.

More recently, BKV has been recovered from urine of bone marrow transplant patients who developed cystitis and may cause ureteral obstruction in renal transplant patients. Importantly, several reports indicate that BKV may be an important cause of interstitial nephritis and associated nephropathy in patients posttransplantation (kidney). Up to 5% of renal allograft recipients can be affected about 40 weeks (range 6-150) posttransplantation. PCR analysis of BKV DNA in the serum is the most widely used blood test for the laboratory diagnosis of BKV-associated nephropathy. Importantly, the presence of BKV DNA in blood reflects the dynamics of the disease: the conversion of plasma from negative to positive for BKV DNA after transplantation, the presence of DNA in plasma in conjunction with the persistence of nephropathy, and its disappearance from plasma after the reduction of immunosuppressive therapy.

The LightCycler PCR detects the 2 polyomaviruses, JCV and BKV; the 2 strains can be differentiated by using the melting curve feature of the instrument. Presently, the qualitative detection of BKV from urine and plasma specimens is reported.

**Useful For**

As a prospective and diagnostic marker for the development of BKV nephropathy in renal transplant recipients.

**Interpretation**

Increasing copy levels of BKV DNA in serial specimens may indicate possible nephropathy in kidney transplant patients.
Cautions

Serial determination of urine specimens from patients may be necessary to monitor increasing (risk of development of nephropathy) or decreasing (treatment efficacy) levels of BK DNA.

References


Method

Viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from clinical specimens. Primers directed to the VP2 gene, which is a conserved sequence specific for JCV and BKV, produce a 131 bp amplicon. The LightCycler instrument (Roche Applied Science) amplifies and monitors the development of target nucleic acid sequences after the annealing step during PCR cycling. This automated PCR system can rapidly detect (30-40 minutes) amplicon development through stringent air-controlled temperature cycling and capillary cuvettes. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3’-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5’-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. Quantitative standards are used to develop a standard curve. Specimens with unknown levels of BKV DNA are then compared to the standard curve to determine the copy level of the virus. (Whiley DM, Mackay IM, Sloots TP: Detection and differentiation of human polyomaviruses JC and BK by LightCycler PCR. J Clin Microbiol, 2001 Dec;39[12]:4357-4361)

Specimen Required:
For optimal results, specimen should arrive within 48 hours of collection.
1.0 mL from a random urine collection. No preservative. Send specimen refrigerated in a 13-mL urine tube. Specimen should arrive within 48 hours for optimal results.

Note: If ordering electronically, no form is required with the specimen. If not ordering electronically, please complete and submit a “Microbiology Request Form” (Supply T244) with the specimen.

Reference Values:
None detected

Analytic Time:
Same day/1 day

Days Set Up:
Monday through Sunday; Varies

CPT Code:
87799
Hantavirus Antibodies, IgG and IgM, Serum #86217

**Profile Information**

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Reporting Name</th>
<th>Available Separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>86161</td>
<td>Hantavirus, IgG, S</td>
<td>No</td>
</tr>
<tr>
<td>86218</td>
<td>Hantavirus, IgM, S</td>
<td>No</td>
</tr>
</tbody>
</table>

**Clinical**

Hantaviruses are emerging pathogens that are distributed worldwide. These RNA-enveloped viruses are members of the family Bunyaviridae and grouped into a separate genus. There are several serotypes of hantavirus, including Hantaan (HTN), Seoul (SEO), Puumala (PUU), Dobrava (DOB), and Sin Nombre.

The virus may have coevolved with specific rodent species as its natural reservoir. Transmission of hantavirus occurs by human contact with deer mouse excreta. 

Hantavirus has caused infections in humans in Eurasia, manifested as hemorrhagic fever with renal syndrome (HFRS), and is characterized by renal failure, hemorrhages, and shock. In the United States, hantavirus infection was first recognized in the Four Corners region (Arizona, New Mexico, Utah, and Colorado) and caused a pulmonary syndrome (hantavirus pulmonary syndrome; [HPS]) in 1993. HPS is characterized by fever and severe pulmonary dysfunction that may be due to host-related immune mechanisms rather than direct viral cytopathology. HPS may have a fatality rate of nearly 50%. In the United States, Sin Nombre virus predominates.

Laboratory diagnosis can be obtained by serologic studies that detect antibody directed to a recombinant nucleocapsid protein of the virus. IgG class antibodies can be detected within 2-8 days after disease onset and remain detectable in sera from the patient for at least 2-3 years. IgM is detectable within 2-8 days of disease onset with almost all patients testing positive between 5-15 days.

**Useful For**

Aiding in the diagnosis of hantavirus infection
### Interpretation

<table>
<thead>
<tr>
<th>IgG</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.900</td>
<td>Negative. This indicates no IgG antibodies to hantavirus were detected.</td>
</tr>
<tr>
<td>0.900-1.100</td>
<td>Equivocal. If clinically indicated, a second serum specimen should be submitted for repeat testing in 2-3 weeks.</td>
</tr>
<tr>
<td>&gt;1.100</td>
<td>Positive. This is presumptive for the presence of IgG antibodies to hantavirus and indicates exposure or infection at some time.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IgM</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.900</td>
<td>Negative. This indicates no IgM antibodies to hantavirus were detected.</td>
</tr>
<tr>
<td>0.900-1.100</td>
<td>Equivocal. If clinically indicated, a second serum specimen should be submitted for repeat testing in 2-3 weeks.</td>
</tr>
<tr>
<td>&gt;1.100</td>
<td>Positive. This indicates the presence of IgM antibodies to hantavirus and is indicative of early response to infection.</td>
</tr>
</tbody>
</table>

### Cautions

- All results from this and other serologies must be correlated with the clinical history, epidemiological data, and other data available.
- Patients with early hantavirus infection may test negative for IgG and IgM antibodies. A negative response does not rule out hantavirus infection. If a negative test is obtained on a patient with signs and symptoms of infection with hantavirus, repeat testing on a second specimen 2-3 weeks later is recommended.
- Specimens from patients with IgM antibodies to cytomegalovirus, influenza virus, and mycoplasma may give positive or equivocal results. If these diseases are suspected, specific serological testing should be carried out.

### References

1. Package Insert, Hantavirus IgG DxSelect (TM)(English) Enzyme-Linked Immunosorbent Assay (ELISA) Product Code EL1600G. Focus Diagnostics, Cypress, California

### Method

**IgG:**
Polystyrene microwells are coated with hantavirus antigens. Diluted serum samples and controls are incubated in the wells to allow specific antibody present in the sample to react with the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated antihuman IgG is added and reacts with specific IgG. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added and the color is allowed to develop. After adding the stop reagent, the resulting color change is quantified by a spectrophotometric reading of optical density (OD), which is directly proportional to the amount of antigen-specific IgG present in the sample. Sample OD readings are compared with reference cutoff OD readings to determine results. (Packet Insert: Hantavirus IgG DxSelect, Focus Diagnostics, Cypress, CA)
IgM:
In the IgM assay the polystyrene microwells are coated with hantavirus antigens. Patient sera and controls are
diluted in a solution containing hyperimmune antihuman IgG precipitating immunoglobulin to remove both free
and complexed IgG from the sample. The diluted serum samples and controls are incubated in the wells to allow
any specific antibody in the samples to react with the antigen. Nonspecific reactants are removed by washing and
peroxidase-conjugated antihuman IgM is added to react with the IgM present. Excess conjugate is removed by
washing. Enzyme substrate and chromogen are added and the color is allowed to develop. After adding the stop
reagent, the resulting color change is quantified by a spectrophotometric reading of optical density (OD), which is
directly proportional to the amount of antigen-specific IgG present in the sample. Sample OD readings are
compared with reference cutoff OD readings to determine results. (Packet Insert: Hantavirus IgM DxSelect, Focus
Diagnostics, Cypress, CA)

**Specimen Required:**
Draw blood in a plain, red-top tube(s). *(Serum gel
tube is acceptable).* Spin down and send 1.0 mL of
serum refrigerated in a screw-capped, round-bottom
vial. Forward promptly.

**Reference Values:**
IgG: <0.90
IgM: <0.90

**Analytic Time:**
Same day/1 day

**Days Set Up:**
Monday through Saturday; 9 am

**CPT Code:**
86790/x2 Virus

**Test Classification:**
FOR RESEARCH USE ONLY. Performance
characteristics have been determined by (Laboratory
Medicine and Pathology, Mayo Clinic, Rochester,
MN). Results should be interpreted in conjunction
with clinical findings.
Influenza is usually a mild illness of the upper respiratory tract resulting in malaise and headache, followed by abrupt onset of fever, severe myalgia, and usually a nonproductive cough. Influenza virus infection can lead to more serious complications such as:

- Combined influenza and bacterial pneumonia
- Primary influenza virus pneumonia
- Severe complications in patients with preexisting conditions such as rheumatic heart disease, broncho-pulmonary disease, impaired renal function, and immunocompromised patients
- Serious illness during the first 2 years of life, with croup, bronchitis, and pneumonia

Type A viruses are typically associated with the most serious influenza epidemics, while type B viruses typically cause milder infections than type A.

The prevalence of influenza varies from year to year, with outbreaks typically occurring during the fall and winter months.

**Useful For**

Rapid detection of influenza virus types A and B in upper respiratory tract specimens

**Interpretation**

Positive results are diagnostic of influenza A or influenza B.

Negative results do not rule out infection with influenza virus.

**Cautions**

- Results will vary depending on socioeconomic status, age of population, geographic location, time of year, and specimen collection/handling.
- The sensitivity of the assay is very dependent upon the quality of the specimen submitted.
- The test is specific for influenza A and B; therefore, the results do not eliminate the possibility of infection with other viruses.
- A negative test does not exclude infection with influenza A or influenza B virus. Therefore, the results obtained should be used in conjunction with clinical findings to make an accurate diagnosis.
Cautions cont.

- This assay detects both viable and nonviable virus. Test performance depends on viral load in the specimen and may not correlate with cell culture performed on the same specimen.
- Performance of the assay has not been established for monitoring antiviral treatment of influenza.
- Use of transport media will result in dilution of samples, which may lower overall test sensitivity.

References


Method

The Light Cycler PCR has been optimized to detect common conserved sequences in the matrix genes of influenza virus type A and influenza virus type B.

Viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from respiratory specimens. Primers directed to the matrix gene amplify a specific sequence of the virus. For the test, influenza virus type A and influenza virus type B genomic RNA is transcribed to cDNA. The LightCycler instrument (Roche Applied Science) amplifies and monitors the development of target nucleic acid sequences after the annealing step during PCR cycling by fluorescence assay. This automated PCR system can rapidly (about 1 hour) detect amplicon development through stringent air-controlled temperature cycling and capillary cuvettes. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3'-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5'-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. Analysis of the PCR amplification and probe melting curves is accomplished through the use of LightCycler software. (Cockerill FR III, Uhl JR: Applications and challenges of real-time PCR for the clinical microbiology laboratory. In Rapid Cycle Real-Time PCR Methods and Applications. Edited by U Reischel, C Wittwer, F Cockerill. Berlin, Germany, Springer-Verlag; 2002, pp 3-30)

Specimen Required:
Nasopharyngeal Aspirate or Washing
Submit greater than 1.5 mL of nasopharyngeal aspirate or washing, refrigerated in a screw-capped, sterile container. Maintain sterility and forward promptly.

Nasopharyngeal Swab (Rayon Mini-Tip Swab) or Throat Swab
Submit a nasopharyngeal swab (rayon mini-tip swab) or throat swab refrigerated. (Calcium alginate-tipped swab or transport swab containing gel is not acceptable for PCR testing.) Maintain sterility and forward promptly.

Note: 1. Specimen source is required on request form for processing.
2. If ordering electronically, no form is required with the specimen. If not ordering electronically, please complete and submit a “Microbiology Request Form” (Supply T244) with the specimen.

Reference Values:
Negative for influenza A nucleic acid
If positive, reported as Influenza A nucleic acid detected

Negative for influenza B nucleic acid
If positive, reported as Influenza B nucleic acid detected

Analytic Time:
Same day/1 day

Days Set Up:
Monday through Sunday

CPT Code: 87798/x2
Clinical

Iron uptake into cells is mediated through internalizing iron-transferrin complexes. The iron-transferrin complex binds to transferrin receptors present on the external face of the plasma membrane, and is internalized through endosomes with ultimate release of iron into the cytoplasm. Plasma membrane-bound transferrin receptor is released by proteolytic cleavage of the extracellular domain, resulting in the formation of a truncated soluble transferrin receptor (sTfR) that circulates freely in the blood.

The concentration of sTfR is an indicator of iron status. Iron deficiency causes overexpression of transferrin receptor and sTfR levels, while iron repletion results in decreased sTfR levels. While ferritin measurement is the accepted method for assessment of iron deficiency, ferritin is an acute-phase reactant and elevates in response to processes that do not correlate with iron status, including inflammation, chronic disease, malignancy, and infection. sTfR is not an acute-phase reactant and the interpretation of iron status using sTfR measurement is not affected by these confounding pathologies.

Useful For

Evaluation of suspected iron deficiency in patients who may have inflammation, infection, or chronic disease and other conditions in which ferritin concentration does not correlate with iron status, including:
- Cystic fibrosis patients who frequently have inflammation or infections
- Evaluating insulin-dependent diabetics who may have iron deficiency resulting from gastric autoimmunity and atrophic gastritis

Interpretation

sTfR concentrations are inversely related to iron status; sTfR elevates in response to iron deficiency and decreases in response to iron repletion.

Cautions

- The sTfR immunoassay should not be used for the routine clinical evaluation of patients for iron status when ferritin immunoassay (#8689 “Ferritin, Serum”) would be appropriate, such as in the absence of confounding pathologies (inflammation, infection, chronic disease, or malignancy).
- Patients with hemolysis and recent blood loss may have falsely elevated sTfR levels.
- sTfR is elevated in patients with thalassemia and sickle cell disease. Caution should be exercised in managing anemia in these individuals based on the sTfR test results.
Specimen Required:
Draw blood in serum gel tube(s). Spin down and send 0.5 mL of serum frozen in plastic vial.

Reference Values:
1.8-4.6 mg/L
It is reported that African Americans may have slightly higher values.

Analytic Time:
Same day/1 day

Days Set Up:
Monday through Sunday; Continuously

CPT Code:
84238

References

Method
Latex-bound anti-sTfR antibodies react with the antigen in the sample to form an antigen/antibody complex. Following agglutination, the complex is measured turbidimetrically on a Roche P Modular. (The Tina-quant Soluble Transferrin Receptor Immunoturbidimetric assay for the in vitro quantitative determination of soluble transferrin receptor. Roche Corporation, Indianapolis, IN 46250, 2001)
Clinical

Following primary metabolism by the phase I enzymes (by oxidation, reduction, dealkylation, and cleavage in the intestines and liver), many drugs and their metabolites are further modified for excretion by a group of conjugative, phase II enzymes. One of these phase II enzymes, uridine diphosphate (UDP)-glycuronosyl transferase 1A1 (UGT1A1), is responsible for bilirubin conjugation with glucuronic acid. This renders the bilirubin water soluble and permits excretion of the bilirubin-glucuronide conjugates in urine. Partial deficiency of UGT1A1 manifests as Gilbert syndrome, a mild familial unconjugated hyperbilirubinemia that may present only under stress. More severe deficiencies of UGT1A1 manifest as Crigler-Najjar syndromes I and II with markedly greater degrees of unconjugated hyperbilirubinemia.

UGT1A1 is also involved in the metabolism of irinotecan, a topoisomerase I inhibitor. Irinotecan is a chemotherapy drug useful in treating solid tumors including colon, rectal, and lung cancers. It is a prodrug that forms an active metabolite, SN-38. SN-38 is normally inactivated by conjugation with glucuronic acid followed by biliary excretion into the gastrointestinal tract. If UGT1A1 activity is impaired or deficient, SN-38 fails to become conjugated with glucuronic acid, increasing the load concentrations of SN-38. This can result in severe neutropenia. The combination of neutropenia with diarrhea can be life threatening.

The most common cause of irinotecan-induced neutropenia results from insertion of extra TA (thymine, adenine) repeats in the TATAA box of the UGT1A1 promoter. This results in decreased expression of the UGT1A1 gene in individuals homozygous for these promoter polymorphisms, and is the most frequent cause of Gilbert syndrome. The number of TA repeats is inversely related to gene expression. Individuals with normal levels of UGT1A1 expression have 6 copies of the TA repeat in the promoter (referred to as the *1 allele) or more rarely 5 copies of the TA repeat (referred to as *36). Individuals with decreased expression of UGT1A1 have 7 TA repeats (*28 allele) or 8 TA repeats (*37). Approximately 10%-15% of Caucasians and African Americans are homozygous for the TA7 repeat (*28/*28), and these individuals have a 50% higher risk of experiencing severe (grade 4 or 5) neutropenia following the administration of irinotecan. Approximately 40% of individuals treated with irinotecan are heterozygous for the TA7 repeat polymorphism (ie, TA6/TA7 or *1/*28). These individuals are also at increased risk of grade 4 neutropenia. Recently, the package labeling for irinotecan has been changed to indicate that individuals homozygous or heterozygous for polymorphisms present in the TATAA box of the UGT1A1 promoter have a higher risk for severe or life-threatening neutropenia. It appears that the risk is greatest for individuals who receive irinotecan once every 3 weeks.
Useful For

- Identifying individuals who are at increased risk of adverse drug reactions with irinotecan and who should be considered for decreased dosing of the drug
- Confirmation of a diagnosis of Gilbert syndrome

Interpretation

An interpretive report will be provided.

Drug-drug interactions must be considered when predicting the UGT1A1 phenotype, especially in individuals heterozygous for the TA7 polymorphism (see “Cautions”).

Cautions

- This test does not detect polymorphisms other than *1, *28, *36, and *37. Numerous polymorphisms and rare mutations have been described that impair UGT1A1 activity.
- Liver or renal dysfunction may result in adverse drug reactions with irinotecan independently of TA repeat polymorphisms.
- Drugs that significantly inhibit cytochrome P450 3A4, such as ketoconazole, increase patient exposure to irinotecan and its active metabolite SN-38, potentially causing or increasing the severity of an adverse drug reaction. The package labeling should be consulted for additional information.
- Drugs that induce the overexpression of cytochrome P450 3A4, including anticonvulsant medications (such as phenytoin, phenobarbital, and carbamazepine), and rifampin, will cause substantial reduction in exposure to irinotecan and its active metabolite SN-38. The package labeling should be consulted for changes to the use of other medications.
- Herbal supplements that induce the overexpression of cytochrome P450 3A4 such as St. John’s Wort, will cause substantial reduction in exposure to irinotecan and its active metabolite SN-38. The package labeling should be consulted for changes to the use of other medications.

References


Method

The promoter of UGT1A1 is amplified by polymerase chain reaction (PCR). The amplified promoter is directly sequenced for the TATAA box region to determine the number of TA repeats. (Guillamette C: Pharmacogenomics of human UDP-glucuronosyl transferase enzymes. Pharmacogenomics 2003;3:136-158)
Specimen Required:
Draw blood in a lavender-top (EDTA) tube(s), and send 2.0 mL of EDTA whole blood in original VACUTAINER(S).

Reference Values:
An interpretive report will be provided.

Analytic Time:
2 days

Days Set Up:
Monday through Friday; 12 pm

CPT Code:
“UGT1A1 TA Repeat Genotype”
83892/Enzyme digestion
83894/Electrophoresis
83898/Amplification of nucleic acid, each primer pair
83912/Interpretation and report

“Extraction DNA-PCR Quality”
83890/Molecular isolation or extraction

“UGT1A1 Gene, Sequencing”
83904/x2 Sequencing