Biliary Tract Malignancy, Fluorescence In Situ Hybridization (FISH) #81048

Clinical
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.

Fluorescence in situ hybridization (FISH) utilizes fluorescently labeled DNA probes to examine cells for chromosomal alterations. FISH can be used to detect cells with chromosomal changes (eg, aneuploidy) that are indicative of malignancy. Studies in our laboratory indicate that the sensitivity of FISH to detect malignant cells in biliary brush and bile aspirate specimens is superior to that of conventional cytology.

Useful For
Assessing bile duct brushing and bile aspirate specimens for malignancy

Interpretation
• Significant populations of cells with chromosomal gains indicate that the patient has a primary or metastatic biliary tract malignancy.
• Positive: 5 or more cells with gains of 2 or more chromosomes, or 10 or more cells with a gain of a single chromosome
• Negative: cases not fulfilling the above criteria for positivity

Cautions
A positive FISH result does not identify location or type of malignancy. Cytology and biopsy may help clarify such situations.

Supportive Data
Bile duct brushing and bile aspirate specimens were collected from 131 patients at the time of ERCP. Cytological specimens from these patients were evaluated for malignancy with FISH and exfoliative cytology. Among patients with biopsy-proven malignancy at the time of ERCP, the sensitivity of FISH was superior to cytology (77% vs. 31%, $P=0.02$). The specificity of FISH and cytology were similar (95% vs. 100%).

References

Method
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 (Vysis UroVysion, Abbott Laboratories, Waukegan, IL) are hybridized to the cells on the slide. 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 (eg, 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.
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.

Submit only 1 of the following specimens:

Aspirate
Send ≥5.0 mL of bile aspirate in a cytology-based fixative (PreservCyt or CytoLyt recommended, but other types of fixatives are acceptable).

Duct Brushings
Send ≥5.0 mL of bile duct brushings in a cytology-based fixative (PreservCyt or CytoLyt recommended, but other types of fixatives are acceptable).

Reference Values: An interpretive report will be provided.
Analytic Time: 4 days
Days Set Up: Monday through Friday
Fee: $847.30
CPT Code: 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.
CPT Code Information
88271/x4 DNA probe, each
88274/Interphase in situ hybridization, analyze 100-300 cells
88291/Interpretation and report
Congenital Adrenal Hyperplasia (CAH)
Newborn Screening, Blood Spot
#84113

Clinical
Congenital adrenal hyperplasia (CAH) is caused by inherited defects in steroid biosynthesis, in particular 21-hydroxylase deficiency. The resulting hormone imbalances with reduced glucocorticoids and mineralocorticoids, and elevated 17-hydroxyprogesterone (17-OHP) and androgens, can lead to life-threatening salt-wasting crises in the newborn period and incorrect gender assignment of virilized females. Hormone replacement therapy, when initiated early, enables a significant reduction in morbidity and mortality. Therefore, newborn screening for CAH is desirable and has been implemented in 37 states. Immunoassays are typically used to quantify 17-OHP as a marker for CAH. However, these immunoassays are hampered by cross-reactivity of the antibodies with other steroids, yielding a high rate of false-positive results. Patients with CAH have elevated androstenedione in addition to low or absent cortisol. Tandem mass spectrometry (MS/MS) allows for the simultaneous specific determination of 17-OHP and other steroids, such as androstenedione and cortisol. Application of this technology to the analysis of newborn screening blood spots significantly enhances the correct identification of patients with CAH and reduces the number of false-positive screening results.

Useful For
Second-tier testing of newborns with abnormal screening result for CAH

Interpretation
Findings of elevated 17-OHP values (controls: <12.5 ng/mL) and a high (17-OHP + androstenedione)/cortisol ratio (controls: <3.75) are supportive of the initial abnormal newborn screening result. Clinical and laboratory follow-up is strongly recommended.

Cautions
This is a screening test and, while its positive predictive value is significantly higher than that of immunoassays (9% vs. 0.5%), false-positive results can occur. Follow-up of abnormal results is necessary; perform #9231 "17-Hydroxyprogesterone, Serum" and #8547 "Deoxycorticosteroids, Plasma" on a serum specimen.

References

Method
One 3/16" disc is punched from a dried blood spot and placed in a glass tube. A solution containing stable isotope labeled 17-OHP is added to each tube. The 17-OHP, androstenedione, and cortisol are eluted from the dried blood spot discs into the liquid solution, which is extracted with diethyl ether. The ether fraction is transferred to a clean tube, and evaporated in a warm-water bath using nitrogen. The residue is redissolved in liquid chromatography (LC) mobile phase and transferred to autosampler vials. Analysis is by electrospray LC-MS/MS operating in the positive mode and using selected reaction monitoring. Quantification is based on calibration curves in ratio to the internal standard. (Minutti C, Magera MJ, Casetta BN, et al: Analysis of 17-OH progesterone [17OHP] by tandem mass spectrometry [MS/MS] for the detection of congenital adrenal hyperplasia [CAH] in newborn blood spots. J Inherit Metab Dis 2001;24 [Suppl 1]:10).
Test Title: Congenital Adrenal Hyperplasia (CAH) Newborn Screening, Blood Spot #84113

<table>
<thead>
<tr>
<th>Specimen Required:</th>
<th>2 blood spots from local newborn screening card (at least 1 spot should be complete, unpunched). If collection of a new specimen is necessary, let blood dry on the supplied filter paper (Supply T493) at ambient temperature in a horizontal position for 3 hours.</th>
</tr>
</thead>
</table>
| Note:              | 1. Patient must be less <1 month of age.  
                       2. Do not expose specimen to heat or direct sunlight.  
                       3. Do not stack wet specimens.  
                       4. Do not use devices or capillary tubes to collect specimen.  
                       5. Keep specimen dry.                                                                                                           |
| Reference Values:  | An interpretive report will be provided.                                                                                                                                                                                                                     |
| Analytic Time:     | 1 day                                                                                                                                                                                                                                                       |
| Days Set Up:       | Monday through Friday                                                                                                                                                                                                                                |
| Fee:               | $35.00                                                                                                                                                                                          |
| CPT Code:          | 83788
Neurologic Enzyme Evaluation
#84162

Profile Information

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<th>Unit Codes</th>
<th>Reporting Title</th>
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<td>Neurologic Enzymes Interpretation</td>
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<tr>
<td>2635</td>
<td>Glutathione, B</td>
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<tr>
<td>80188</td>
<td>Phosphofructokinase, RBC</td>
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<tr>
<td>2653</td>
<td>Phosphoglycerate Kinase, B</td>
<td>No</td>
</tr>
<tr>
<td>2633</td>
<td>Triosephosphate Isomerase, B</td>
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Clinical

Several red blood cell enzymes are known to cause a nonspherocytic hemolytic anemia (HA). The most common cause of these are glucose-6-phosphate dehydrogenase (G-6-PD) and pyruvate kinase (PK) deficiency. Four other RBC enzymes that cause HA have also been associated with hereditary myopathic or neurologic disorders. These enzymes are phosphofructokinase (PFK), triosephosphate isomerase (TPI), phosphoglycerate kinase (PGK), and glutathione synthase. Kinetic enzyme assays are available for the first 3 disorders. Quantitative measurement of glutathione substitutes for analysis of the enzyme glutathione synthase.

Useful For

Evaluating patients who have a hemolytic process that is associated with some neurologic findings

Interpretation

- Definitive results and an interpretive report will be provided.
- Significant abnormal values typically are 25% of values obtained for a normal individual.

Cautions

No significant cautionary statements.

References


Method

See Individual Unit Codes.

Specimen Required:

Draw blood in a yellow-top (ACD) tube(s), and send 10 mL of ACD whole blood refrigerated. SPECIMEN CANNOT BE FROZEN. Do not transfer blood to other tubes.

Reference Values:

Definitive results and an interpretive report will be provided.

Analytic Time: 10 days
Days Set Up: Monday through Friday
Fee: $1,194.60
CPT Code: 80500/Clinical pathology consultation, limited
82657/RBC enzymes
82978/Glutathione
New Test ANNOUNCEMENT

Extended Cardiovascular Risk Marker Panel

#84208

Profile Information

<table>
<thead>
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<th>Available separately</th>
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<td>8484</td>
<td>Fibrinogen, P</td>
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<tr>
<td>80379</td>
<td>Homocysteine, Total, P</td>
<td>Yes</td>
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<tr>
<td>81558</td>
<td>Lipoprotein a, S</td>
<td>Yes</td>
</tr>
<tr>
<td>81913</td>
<td>LDL Subfractionation, P</td>
<td>Yes</td>
</tr>
<tr>
<td>82047</td>
<td>C-Reactive Protein-Cardiac, S</td>
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<td>23543</td>
<td>Cholesterol, Total CDC, S</td>
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<td>25341</td>
<td>Triglycerides, CDC, S</td>
<td>Yes (order #8316)</td>
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<td>23540</td>
<td>Cholesterol, HDL, CDC, S</td>
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<td>23542</td>
<td>Calculated LDL</td>
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<tr>
<td>23526</td>
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Clinical

Cardiovascular disease is the number one cause of death in the United States. Of those individuals who experience an ischemic event (estimated 1.5 million heart attacks and 0.5 million strokes), fully one third will die as a result of that event. Many of the individuals who have an event have no prior symptoms. Prevention of ischemic cardiovascular events is key.

Risk factors, including age, smoking status, hypertension, diabetes, cholesterol, and high-density lipoprotein (HDL) cholesterol, are used by physicians to identify individuals likely to have an ischemic event. However, current risk factors predict only about 65% of individuals who will go on to have a cardiovascular event. Additional risk markers have been identified for cardiovascular disease. Many markers have been suggested. A few have emerged as independent risk markers, but none have been proven to be causative. These include homocysteine, C-reactive protein, lipoprotein a (Lp[a]), fibrinogen, and small, dense low-density lipoproteins (LDL). The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) identified these novel markers as "emerging risk factors" and indicated that clinicians may utilize them in selected persons to guide intensity of risk-reduction therapy and modulate clinical judgement when making therapeutic decision.1 Prospective studies assessing these risk factors individually have determined them to be independently associated with increased risk for the development of ischemic events, although not all studies have consistently demonstrated this.2-6

The ATP-III did not identify in which group of patients these markers are best used or how to respond to elevated values. We suggest that analysis of additional risk markers is most appropriate in individuals who are at intermediate risk for developing cardiovascular disease, as determined using the Framingham 10-year risk score of 10-20%. Some have suggested using 6-10% as defining the intermediate risk group.

Useful For

- Assessment of risk of developing myocardial infarction in patients presenting with acute coronary syndromes
- Assessment of risk of developing cardiovascular disease or ischemic events in individuals who do not have manifest disease at present

Interpretation

- An interpretative report will be included with the results.
- More aggressive treatment strategies may be pursued in patients determined to be at increased risk.
- A Framingham risk score will also be provided, but requires information about the patient including age, gender, smoking status (smoker vs nonsmoker), systolic blood pressure, and whether the patient is being treated with antihypertension medication (treated or untreated).
- See individual unit codes for additional interpretive information.
**Specimen Required:** A plasma 3.2% sodium citrate specimen, a plasma EDTA specimen, and a serum specimen are required for this test.

**Plasma for Fibrinogen**
Draw blood in a light blue-top (3.2% sodium citrate) tube(s) from a fasting patient. Spin down and send 1.0 mL of citrate platelet-poor plasma frozen in plastic vial.

**Note:** Label specimen appropriately (plasma for fibrinogen).

**Plasma for Homocysteine, Total, and LDL Subfractionation**
Draw blood in a lavender-top (EDTA) tube(s) from a fasting patient. Spin down, promptly separate plasma from cells, and send 1.0 mL of EDTA plasma frozen in a plastic vial. **Specimen cannot be sent ambient.**

**Note:** 1. Please include the following patient information:
   A. Systolic blood pressure
   B. Blood pressure treated or untreated
   C. Smoker or nonsmoker

2. Label specimen appropriately (plasma for homocysteine, total and LDL subfractionation).

**Serum for Calculated LDL; Cholesterol, Total, CDC; Cholesterol, HDL, CDC; C-Reactive Protein-Cardiac; Lipoprotein a; and Triglycerides, CDC**
Draw blood in a plain, red-top tube(s) following an overnight (12-14 hour) fast. Patient must not consume any alcohol for 24 hours before the specimen is drawn. Spin down and send 2.5 mL of serum frozen in plastic vial.

**Note:** 1. *Patient's age and sex are required* on request form for processing.

2. Label specimen appropriately (serum).
**Test Title:** Extended Cardiovascular Risk Marker Panel
#84208

**Reference Values:**

**FIBRINOGEN**
175-430 mg/dL
In normal, full-term newborns and in healthy, premature infants (30-36 weeks gestation), fibrinogen is near adult levels (>150 mg/dL) and reaches adult levels by ≤21 days postnatal.

**HOMOCYSTEINE, TOTAL**
Adults: ≤13 µmol/L
Reference values apply to fasting specimens only.

**LIPOPROTEIN a**
≤30 mg/dL
Values ≥30 mg/dL may suggest increased risk of coronary heart disease.

**LDL SUBFRACTIONATION**
>26.5 nm: phenotype A (large, buoyant LDL)
26.3-26.5 nm: intermediate LDL
<26.3 nm: phenotype B (small, dense LDL)
Phenotype B (small, dense LDL) has been determined to be associated with an increased risk of coronary artery disease (CAD). Intermediate LDL size may be associated with a heightened risk for developing CAD.

**C-REACTIVE PROTEIN-CARDIAC**
Low risk: <1.0 mg/L
Average risk: 1.0-3.0 mg/L
High risk: >3.0 mg/L
Acute inflammation: >10.0 mg/L

**CHOLESTEROL, TOTAL**
Desirable: <200 mg/dL
Borderline high: 200-239 mg/dL
High cholesterol: ≥240 mg/dL

**CHOLESTEROL, HDL**
Low HDL: <40 mg/dL
Normal: 40-60 mg/dL
Desirable: ≥60 mg/dL

**TRIGLYCERIDES**
Normal: <150 mg/dL
Borderline high: 150-199 mg/dL
High: 200-499 mg/dL
Very high: ≥500 mg/dL

**LDL CHOLESTEROL**
Optimal: <100 mg/dL
Low risk: 100-129 mg/dL
Borderline high: 130-159 mg/dL
High: 160-189 mg/dL
Very high: ≥190 mg/dL

**Analytic Time:** 3 days
**Days Set Up:** Monday through Friday, Sunday
**Fee:** $344.90

**CPT Code:**
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.

**CPT Code:**
81913/LDL Subfractionation, Plasma
80061/lipid panel (includes HDL, total cholesterol, and triglycerides)
83090/Homocysteine, total
83520/Lipoprotein a
83716/LDL subfractionation
85384/Fibrinogen
86141/C-reactive protein-Cardiac
Glutamic Acid Decarboxylase (GAD65) Antibody Assay, Spinal Fluid #84221

With the introduction of this test for GAD65 antibodies in spinal fluid, #81596 Glutamic Acid Decarboxylase (GAD65) Antibody Assay, Serum will only be used for serum specimens.

Clinical

Glutamic acid decarboxylase (GAD) is a neuronal enzyme involved in the synthesis of the neurotransmitter gamma-aminobutyric acid (GABA). Antibodies directed against the 65-kd isoform of GAD (GAD65) are seen in a variety of autoimmune neurologic disorders including stiff-man (Moersch-Woltman) syndrome, autoimmune cerebellitis, myasthenia gravis, Lambert-Eaton syndrome, and some rare acquired nonparaneoplastic encephalomyelopathies.

GAD65 antibodies are also the major pancreatic islet antibodies and are an important serological marker of predisposition to type 1 (insulin-dependent) diabetes. GAD65 autoantibodies also serve as a marker of predisposition to other autoimmune diseases that occur with type 1 diabetes, including autoimmune thyroid disease (e.g., thyrotoxicosis, Graves’ disease, Hashimoto’s thyroiditis, hypothyroidism), pernicious anemia, premature ovarian failure, Addison’s disease (idiopathic adrenocortical failure), and vitiligo.

Useful For

- Assessing susceptibility to autoimmune (type 1, insulin-dependent) diabetes mellitus and related endocrine disorders (e.g., thyroiditis and pernicious anemia)
- Distinguishing between patients with type 1 and type 2 diabetes: assays for gastric parietal cell, thyroglobulin, and thyroid peroxidase antibodies complement GAD65 antibody in this context
- Confirming a diagnosis of stiff-man syndrome and autoimmune cerebellitis

Interpretation

High titers (≥20 nmol/L) are found in classic stiff-man syndrome (93% positive) and in related autoimmune neurologic disorders (e.g., acquired cerebellar ataxia, some acquired nonparaneoplastic encephalomyelopathies).

Diabetic patients with polyendocrine disorders also generally have GAD65 antibody values ≥20 nmol/L.

Values in patients who have type 1 diabetes without a polyendocrine or autoimmune neurologic syndrome are usually <20 nmol/L. Low titers (0.03-19.9 nmol/L) are detectable in the serum of approximately 80% of type 1 diabetic patients. Conversely, low titers are detectable in the serum of approximately <5% of type 2 diabetic patients.

Low titers are found in approximately 25% of patients with myasthenia gravis, Lambert-Eaton syndrome, and rarer autoimmune neurological disorders. Eight percent of miscellaneous neurological disorders in healthy Olmsted County residents over age 50 have low-positive values. These are not false-positive; the antibodies are inhibited by unlabeled GAD65 antigen and are accompanied in at least 50% of cases by related organ-specific autoantibodies.

Values ≥0.03 nmol/L are consistent with susceptibility to autoimmune (type 1) diabetes and related endocrine disorders (thyroiditis and pernicious anemia).

Cautions

Antibodies specific for GAD65 account for most, but not all, antibodies detected in the islet cell antibody test.
**Test Title:** Glutamic Acid Decarboxylase (GAD65) Antibody Assay, Spinal Fluid

**References**

**Method**
(125)I-labeled recombinant human GAD65 is incubated with the patient's diluted serum. Antihuman IgG and IgM are then added to form an immunoprecipitate. After washing the precipitated immune complexes, specific antibodies are detected by counting y-emission from the pellet's bound I-GAD65. (Walikonis JE, Lennon VA: Radioimmunassay for glutamic acid decarboxylase [GAD65] auto-antibodies as a diagnostic aid for stiff-man syndrome and a correlate of susceptibility to type 1 diabetes mellitus. Mayo Clin Proc 1998 December;73[12]:1161-1166)

**Specimen Required:** 1.0 mL of spinal fluid. Send specimen refrigerated.

**Reference Values:** ≤0.02 nmol/L

**Fee:** $151.60

**CPT Code:** 86341
Synthetic Glucocorticoid Screens
#81031 Serum
#81035 Urine
#81089 Medicinal Formulations (Drugs)

Clinical
Synthetic glucocorticoids are widely used and have important clinical utility both as anti-inflammatory and immunosuppressive agents. The medical use of these agents, as well as their surreptitious use, can sometimes lead to a confusing clinical presentation. Patients exposed to these steroids may present with clinical features of Cushing’s syndrome, but with suppressed cortisol levels and evidence of hypothalamus-pituitary-adrenal axis suppression.

Useful For
- Confirming the presence of the listed synthetic glucocorticoids
- Confirming the cause of secondary adrenal insufficiency

Interpretation
This test screens for and quantitates, if present, the following synthetic glucocorticoids: beclomethasone dipropionate, betamethasone, budesonide, dexamethasone, fludrocortisone, flunisolide, fluorometholone, fluticasone propionate, megestrol acetate, methylprednisolone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide.

The presence of synthetic glucocorticoids in urine indicates current or recent use of these compounds. Since several of these compounds exceed the potency of endogenous cortisol by 1 or more orders of magnitude, even trace levels may be associated with Cushingoid features.

Cautions
- This method cannot detect all of the available synthetic steroids either available as pharmaceutical compounds or chemicals present in food. The assay confirms only the listed synthetic glucocorticoids (see Interpretation).
- For serum and urine specimens, lack of detection does not preclude use of the synthetic glucocorticoids, as adrenal suppression may persist for some time after the exogenous steroid is discontinued.

References
Method

The synthetic glucocorticoids are extracted from the specimen (serum, urine, or medicinal formulation) using an acetonitrile protein precipitation followed by methylene chloride liquid extraction of the solvent. Cortisol-9, 11, 12, 12-d4 and triamcinolone-d1 acetonide-d6 are added to each sample before the liquid extraction and serve as the internal standards. Then, 17 µL of the reconstituted sample extract is injected into a high-performance liquid chromatography (HPLC) system and analyzed by tandem mass spectrometry (MS/MS). The mass spectrometer has an electrospray interface and is operated in the multiple-reaction monitoring positive mode. The calibration utilizes a 4-point standard curve over a concentration range of 0-25 µg/dL.


Specimen Required:
- #81031 Serum - Draw blood in a plain, red-top tube(s) or a serum gel tube(s). Spin down, separate from clot, and send 2.0 mL of serum frozen in plastic vial.
- #81035 Urine - 5.0 mL from a random urine collection. No preservative. Send specimen frozen in a plastic, 13-mL urine tube.
- #81089 Drugs - Send 2 tablets or double a typical prescribed dose.

Preferred Specimen Transport:
- #81031 Serum - Frozen
- #81035 Urine - Frozen
- #81089 Drugs - Ambient

Reference Values:

<table>
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<th>Cutoff concentrations</th>
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<tbody>
<tr>
<td>Beclomethasone Dipropionate: 0.03 µg/mL</td>
</tr>
<tr>
<td>Betamethasone: 0.05 µg/mL</td>
</tr>
<tr>
<td>Budesonide: 0.04 µg/mL</td>
</tr>
<tr>
<td>Dexamethasone: 0.06 µg/mL</td>
</tr>
<tr>
<td>Fludrocortisone: 0.06 µg/mL</td>
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<td>Flunisolide: 0.03 µg/mL</td>
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<td>Fluorometholone: 0.04 µg/mL</td>
</tr>
<tr>
<td>Fluticasone Propionate: 0.04 µg/mL</td>
</tr>
<tr>
<td>Megestrol Acetate: 0.03 µg/mL</td>
</tr>
<tr>
<td>Methylprednisolone: 0.06 µg/mL</td>
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<td>Prednisolone: 0.05 µg/mL</td>
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<td>Prednisone: 0.05 µg/mL</td>
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<tr>
<td>Triamcinolone 0.30 µg/mL</td>
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<tr>
<td>Triamcinolone Acetonide: 0.03 µg/mL</td>
</tr>
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</table>

Values for normal patients not taking these synthetic glucocorticoids should be less than the cutoff concentration (detection limit).

Analytic Time: 2 days
Days Set Up: Monday through Friday
Fee: #81031 Serum - $141.02
      #81035 Urine - $143.80
      #81089 Drugs - $197.90
CPT Code: 82544