BioAgilytix Diagnostics

Porphyrins (Total, Fractionation, and Total Reflex)

Specimen Type	Total only: Serum Total or Fractionation: Plasma Total Reflex: Plasma
Specimen Volume	Total Porphyrins: 2 mL Total and Fractionation: 3 mL Total Reflex: 5 mL
Collection	Patient should refrain from alcohol consumption for 24 hours prior to collection. Patient should be off all medications for 1 week prior to sampling. If this is not possible, note all medications on the sample requisition. Plasma: Collect blood in heparinized Sodium or Lithium heparin (green top) tube. Centrifuge and remove plasma from cells as soon as possible. Freeze immediately and protect from light. Serum: Collect blood in red top tube with no anticoagulant. Centrifuge and remove serum from cells as soon as possible. Freeze immediately and protect from light.
Minimum Volume	Total Serum or Plasma: 2 mL Plasma Fractionation: 3 mL
Handling	Ship frozen on dry ice.
Rejection Criteria	• Hemolyzed specimens. • Specimens not protected from light. • Specimens outside of listed stability.
Stability	Frozen for 8 weeks. Protect from light.
Methodology	Fluorimetry for Total. HPLC for Fractionation.
Reference Range	Total Porphyrins: 0.1 – 1.0 µg/dL Plasma Fractionation: < 1.0 µg/dL Individual Porphyrins: < 1.0 µg/dL
Turnaround Time	Porphyrins Total, Plasma: Up to 7 business days. Porphyrins Total, Serum: Up to 7 business days. Porphyrins Fractionation, Plasma: Up to 14 business days.

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Porphyrins Reflex	If the total is elevated, a fractionated porphyrins will be performed. Sample submitted must be plasma and quantity must be sufficient to perform both tests.
CPT Code	Total Porphyrins: 84311 Plasma Porphyrin Fractionation: 82492 x 5
Clinical Significance	The porphyrias are a group of diseases associated with hereditary and acquired deficiencies in the biosynthetic pathway of heme. The laboratory diagnosis of porphyrin disorders rests primarily with the identification of patterns of porphyrin intermediates. Multiple species have been identified for each of the porphyrins based on the order of their substitutions. Porphyrias are disorders of porphyrin metabolism caused by specific defects of enzymes of the heme biosynthetic pathway. Consequently, each type of porphyria is characterized by the accumulation of the precursor proximal to the step dependent on the defective enzyme. All but two porphyrias (acute intermittent and plumbo-porphyria) are characterized by cutaneous lesions caused by the photosensitizing effects of accumulated porphyrins. Maximal skin reactivity occurs in the 400-410 nm region of the spectrum, known as the Soret band, which corresponds to the wavelengths that porphyrins show the most intense absorption. This assay allows differentiation of three conditions according to their porphyrin content. Variegate porphyria, (VP) is an autosomal dominant disorder associated by a defect in the enzyme protoporphyrinogen oxidase, which catalyzes the last step of heme biosynthesis. These cases present a peak in the spectral area of 626-628 nm. Erythropoietic protoporphyria (EPP) is another autosomal dominant condition that causes acute photosensitivity and is characterized by an increase of plasma protoporphyrin with a characteristic peal at 636 nm. A third group which includes normal subjects, non-porphyria patients, and those suffering from acute intermittent porphyria, hereditary coproporphyria, congenital erythropoietic porphyria (Gunther Disease) and porphyria cutaneous porphyria cutanea tarda.
Principle	Plasma or Serum specimens from patients suspected of primary or secondary porphyria are diluted in 1 N hydrochloric acid directly on a microplate. The plate is read by fluorescence at emission 595 nm with 405 nm excitation. Fluorescence response in the sample is proportional to the total porphyrins concentration and is quantified by a standard curve. This test is semi-quantitative with a cut-off value of 1 µg/dL above which total porphyrin levels are said to be elevated. When the levels of total plasma porphyrins are elevated, fractionation is recommended. This method provides quantitative results for uroporphyrin I, heptacarboxyl P.I, hexacarboxyl P.I., pentacarboxyl P.I., copioporphyrin I and protoporphyrin VI. For the fractionation of individual porphyrins, an HPLC method utilizing reversed phase chromatography with a gradient elution is followed by flourometric detection.