The Great Plains Laboratory, LLC

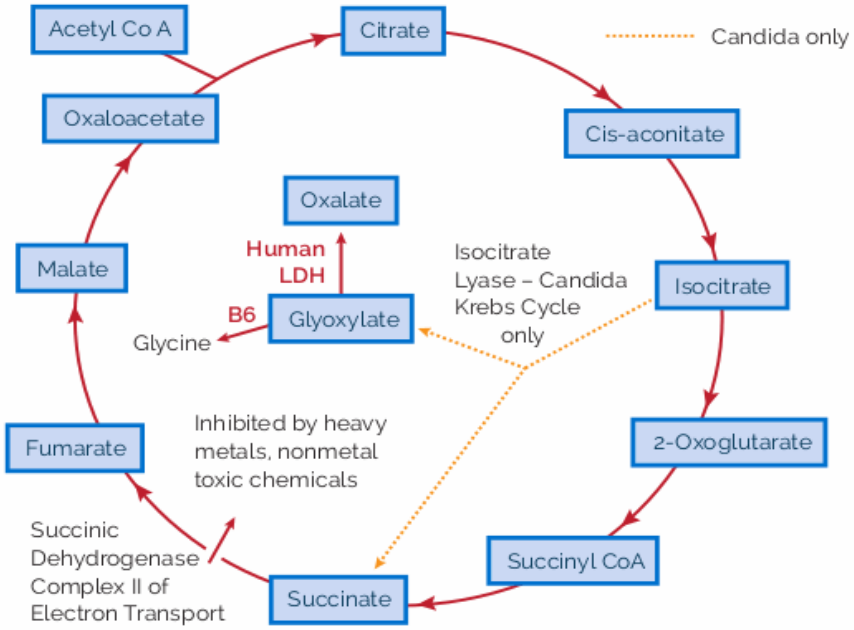
Requisition #:		*Physician:*	
Patient Name:		*Date of Collection:*	
Patient Age:	13	*Time of Collection:*	
Patient Sex:	M	*Print Date:*	

Organic Acids Test - Nutritional and Metabolic Profile

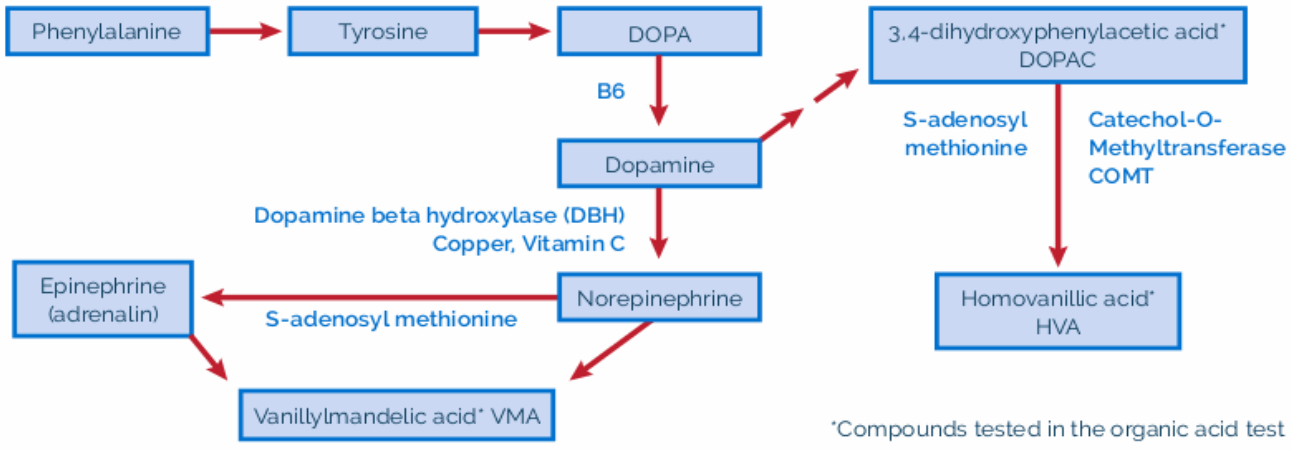
Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Males Age 13 and Over
Intestinal Microbial Overgrowth				
Yeast and Fungal Markers				
1 Citramalic	0.11	- 2.0	H 4.2	4.2
2 5-Hydroxymethyl-2-furoic *(Aspergillus)*	≤ 18		11	11
3 3-Oxoglutaric	≤ 0.11		0	0.00
4 Furan-2,5-dicarboxylic *(Aspergillus)*	≤ 13		7.4	7.4
5 Furancarbonylglycine *(Aspergillus)*	≤ 2.3		0.05	0.05
6 Tartaric *(Aspergillus)*	≤ 5.3		H 814	814
7 Arabinose	≤ 20		H 103	103
8 Carboxycitric	≤ 20		2.1	2.1
9 Tricarballylic *(Fusarium)*	≤ 0.58		0.17	0.17
Bacterial Markers				
10 Hippuric	≤ 241		H 297	297
11 2-Hydroxyphenylacetic	0.03	- 0.47	0.40	0.40
12 4-Hydroxybenzoic	≤ 0.73		0.60	0.60
13 4-Hydroxyhippuric	≤ 14		8.7	8.7
14 DHPPA (Beneficial Bacteria)	≤ 0.23		0.17	0.17
Clostridia Bacterial Markers				
15 4-Hydroxyphenylacetic *(C. difficile, C. stricklandii, C. lituseburense & others)*	≤ 18		10	10
16 HPHPA *(C. sporogenes, C. caloritolerans, C. botulinum & others)*	≤ 102		H 130	130
17 4-Cresol *(C. difficile)*	≤ 39		H 53	53
18 3-Indoleacetic *(C. stricklandii, C. lituseburense, C. subterminale & others)*	≤ 6.8		0.68	0.68

Testing performed by The Great Plains Laboratory, LLC, Lenexa, Kansas. The Great Plains Laboratory has developed and determined the performance characteristics of this test. This test has not been evaluated by the U.S. FDA; the FDA does not currently regulate such testing.

Human Krebs Cycle showing Candida Krebs Cycle variant that causes excess Oxalate via Glyoxylate



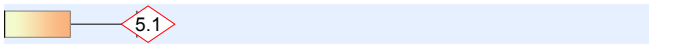
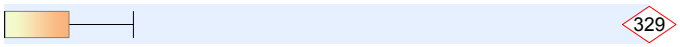
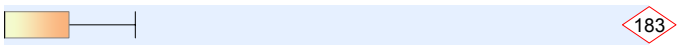
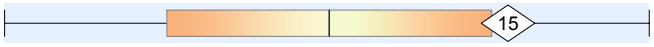
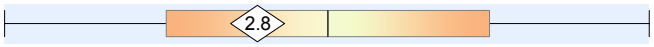
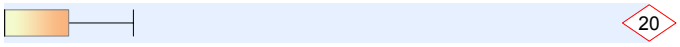
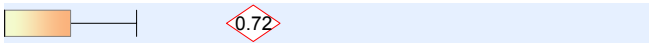
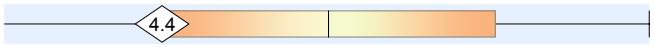
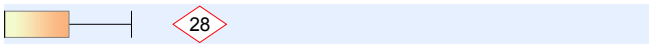
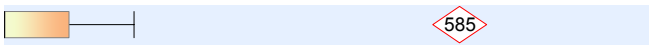
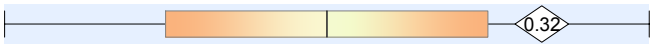
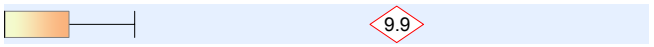
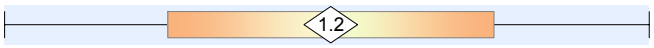
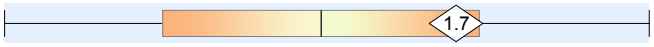
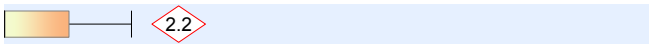
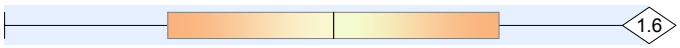
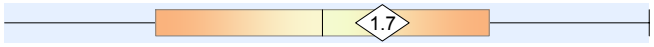
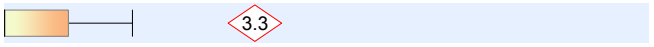
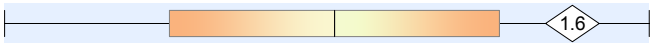
Major pathways in the synthesis and breakdown of **catecholamine neurotransmitters** in the absence of microbial inhibitors



The Great Plains Laboratory, LLC

Requisition #: *Physician:*

Patient Name: *Date of Collection:*

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Males Age 13 and Over
Oxalate Metabolites			
19 Glyceric	0.21 - 4.9	H 5.1	
20 Glycolic	18 - 81	H 329	
21 Oxalic	8.9 - 67	H 183	
Glycolytic Cycle Metabolites			
22 Lactic	0.74 - 19	15	
23 Pyruvic	0.28 - 6.7	2.8	
Mitochondrial Markers - Krebs Cycle Metabolites			
24 Succinic	≤ 5.3	H 20	
25 Fumaric	≤ 0.49	H 0.72	
26 Malic	≤ 1.1	H 2.0	
27 2-Oxoglutaric	≤ 18	4.4	
28 Aconitic	4.1 - 23	H 28	
29 Citric	2.2 - 260	H 585	
Mitochondrial Markers - Amino Acid Metabolites			
30 3-Methylglutaric	0.02 - 0.38	0.32	
31 3-Hydroxyglutaric	≤ 4.6	H 9.9	
32 3-Methylglutaconic	0.38 - 2.0	1.2	
Neurotransmitter Metabolites			
Phenylalanine and Tyrosine Metabolites			
33 Homovanillic (HVA) *(dopamine)*	0.39 - 2.2	H 3.6	
34 Vanillylmandelic (VMA) *(norepinephrine, epinephrine)*	0.53 - 2.2	1.7	
35 HVA / VMA Ratio	0.32 - 1.4	H 2.1	
36 Dihydroxyphenylacetic (DOPAC) *(dopamine)*	0.27 - 1.9	H 2.2	
37 HVA/ DOPAC Ratio	0.17 - 1.6	1.6	
Tryptophan Metabolites			
38 5-Hydroxyindoleacetic (5-HIAA) *(serotonin)*	≤ 2.9	1.7	
39 Quinolinic	0.52 - 2.4	H 3.3	
40 Kynurenic	0.12 - 1.8	1.6	

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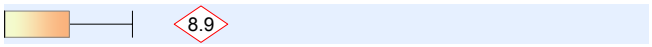
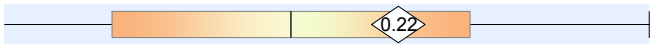
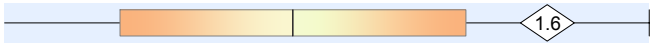
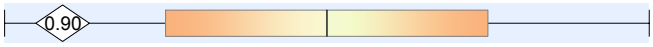
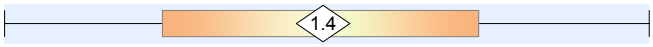
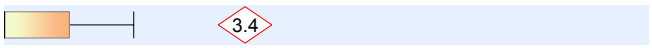
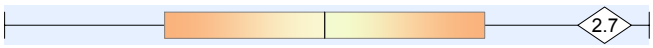
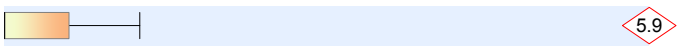
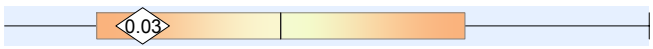
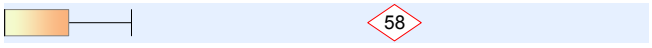
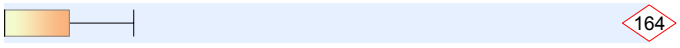
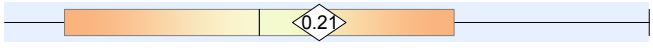
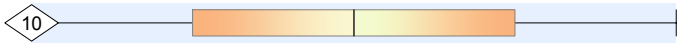
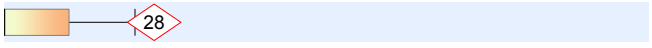
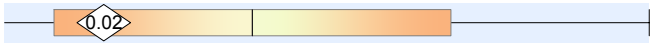
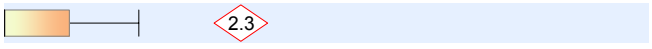
Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Males Age 13 and Over
Pyrimidine Metabolites - Folate Metabolism			
41 Uracil	≤ 6.9	H 8.9	
42 Thymine	≤ 0.36	0.22	
Ketone and Fatty Acid Oxidation			
43 3-Hydroxybutyric	≤ 1.9	1.6	
44 Acetoacetic	≤ 10	0.90	
45 Ethylmalonic	0.13 - 2.7	1.4	
46 Methylsuccinic	≤ 2.3	H 3.4	
47 Adipic	≤ 2.9	2.7	
48 Suberic	≤ 1.9	H 5.9	
49 Sebacic	≤ 0.14	0.03	
Nutritional Markers			
Vitamin B12			
50 Methylmalonic ✱	≤ 2.3	2.2	
Vitamin B6			
51 Pyridoxic (B6)	≤ 26	H 58	
Vitamin B5			
52 Pantothenic (B5)	≤ 5.4	H 164	
Vitamin B2 (Riboflavin)			
53 Glutaric ✱	≤ 0.43	0.21	
Vitamin C			
54 Ascorbic	10 - 200	10	
Vitamin Q10 (CoQ10)			
55 3-Hydroxy-3-methylglutaric ✱	≤ 26	H 28	
Glutathione Precursor and Chelating Agent			
56 N-Acetylcysteine (NAC)	≤ 0.13	0.02	
Biotin (Vitamin H)			
57 Methylcitric ✱	0.15 - 1.7	H 2.3	

✱ ***A high value for this marker may indicate a deficiency of this vitamin.***

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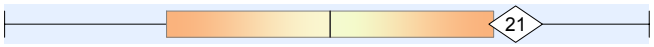
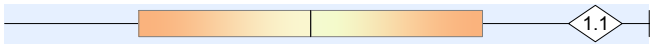
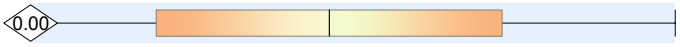
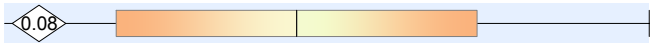
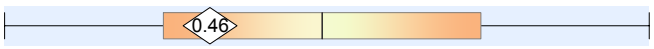
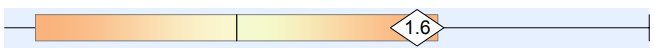
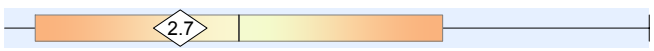
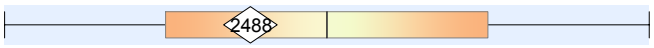
Requisition #: *Physician:*

Patient Name: *Date of Collection:*

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Males Age 13 and Over
Indicators of Detoxification			
Glutathione			
58 Pyroglutamic ✱	5.7 - 25	21	
Methylation, Toxic exposure			
59 2-Hydroxybutyric ✱✱	≤ 1.2	1.1	
Ammonia Excess			
60 Orotic	≤ 0.46	H 0.77	
Aspartame, salicylates, or GI bacteria			
61 2-Hydroxyhippuric	≤ 0.86	H 1.6	
Amino Acid Metabolites			
62 2-Hydroxyisovaleric	≤ 0.41	0	
63 2-Oxoisovaleric	≤ 1.5	0.08	
64 3-Methyl-2-oxovaleric	≤ 0.56	0.53	
65 2-Hydroxyisocaproic	≤ 0.39	0.01	
66 2-Oxoisocaproic	≤ 0.34	0.05	
67 2-Oxo-4-methiolbutyric	≤ 0.14	0	
68 Mandelic	≤ 0.09	0	
69 Phenyllactic	≤ 0.10	0.03	
70 Phenylpyruvic	0.02 - 1.4	0.46	
71 Homogentisic	≤ 0.23	0.02	
72 4-Hydroxyphenyllactic	≤ 0.62	0.24	
73 N-Acetylaspartic	≤ 2.5	1.6	
74 Malonic	≤ 9.9	2.7	
75 4-Hydroxybutyric	≤ 4.3	3.6	
Mineral Metabolism			
76 Phosphoric	1,000 - 4,900	2,488	

✱ *A high value for this marker may indicate a Glutathione deficiency.*

✱✱ *High values may indicate methylation defects and/or toxic exposures.*

Indicator of Fluid Intake

77	*Creatinine	174	mg/dL	

*The creatinine test is performed to adjust metabolic marker results for differences in fluid intake. Urinary creatinine has limited diagnostic value due to variability as a result of recent fluid intake. Samples are rejected if creatinine is below 20 mg/dL unless the client requests results knowing of our rejection criteria.

Explanation of Report Format

The reference ranges for organic acids were established using samples collected from typical individuals of all ages with no known physiological or psychological disorders. The ranges were determined by calculating the mean and standard deviation (SD) and are defined as ± 2SD of the mean. Reference ranges are age and gender specific, consisting of Male Adult (≥13 years), Female Adult (≥13 years), Male Child (<13 years), and Female Child (<13 years).

There are two types of graphical representations of patient values found in the new report format of both the standard Organic Acids Test and the Microbial Organic Acids Test.

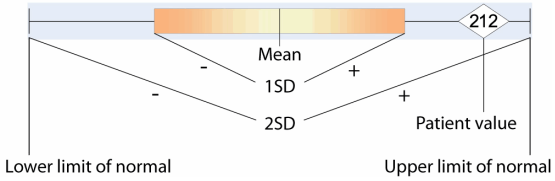
The first graph will occur when the value of the patient is within the reference (normal) range, defined as the mean plus or minus two standard deviations.

The second graph will occur when the value of the patient exceeds the upper limit of normal. In such cases, the graphical reference range is "shrunk" so that the degree of abnormality can be appreciated at a glance. In this case, the lower limits of normal are not shown, only the upper limit of normal is shown.

In both cases, the value of the patient is given to the left of the graph and is repeated on the graph inside a diamond. If the value is within the normal range, the diamond will be outlined in black. If the value is high or low, the diamond will be outlined in red.

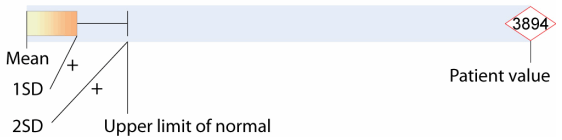
Example of Value Within Reference Range

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Result	Reference Range - Males Age 13 and Under
Intestinal Microbial Overgrowth				
HPHPA (Clostridia marker)	<	219 .9	212	



Example of Elevated Value

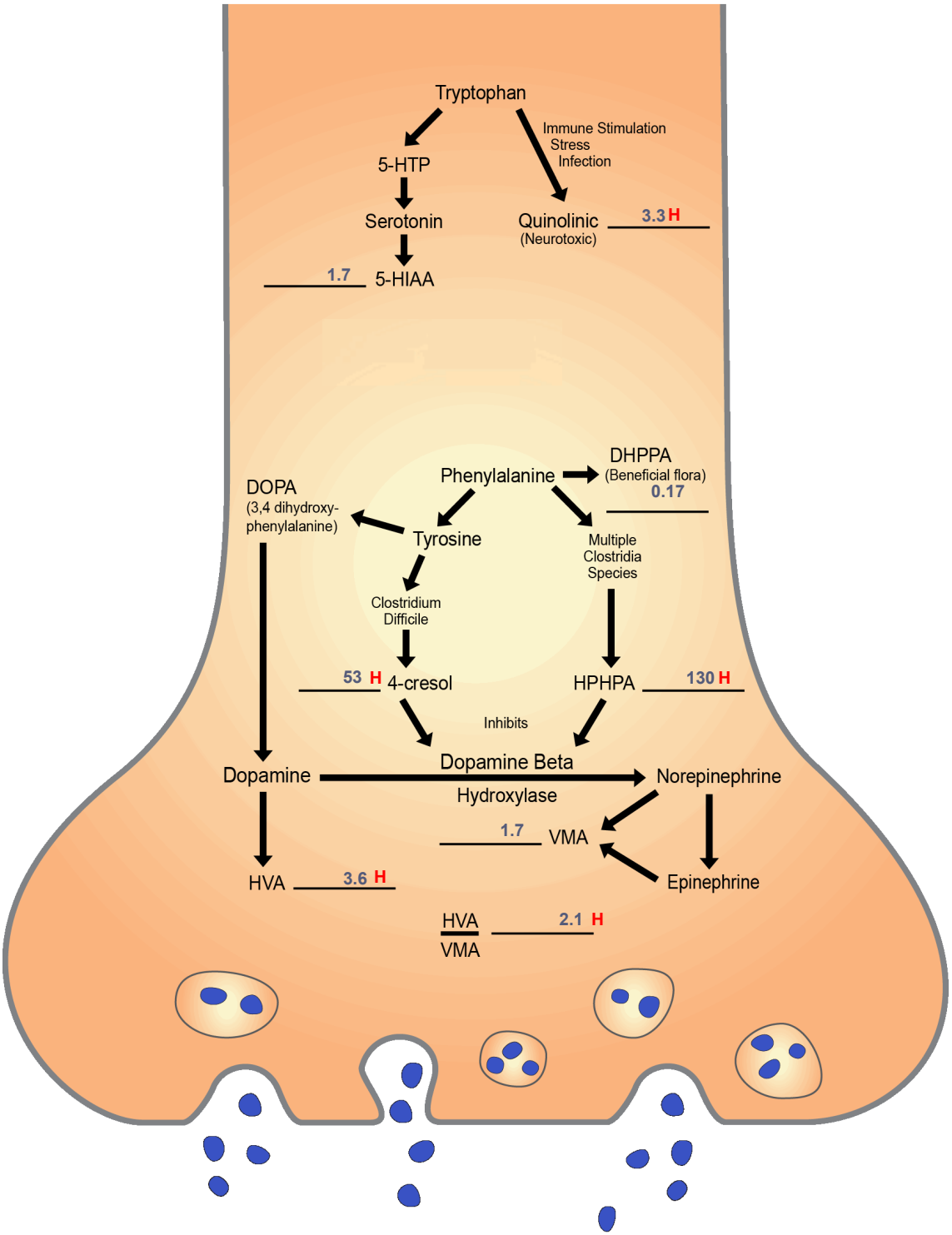
Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Result	Reference Range - Males Age 13 and Under
Intestinal Microbial Overgrowth				
HPHPA (Clostridia marker)	<	219 .9	H 3894	



Requisition #: *Physician:*

Patient Name: *Date of Collection:*

Neurotransmitter Metabolism Markers



The diagram contains the patient's test results for neurotransmitter metabolites and shows their relationship with key biochemical pathways within the axon terminal of nerve cells. The effect of microbial byproducts on the blockage of the conversion of dopamine to norepinephrine is also indicated.

Interpretation

High yeast/fungal metabolites (1-8) Elevations of one or more metabolites indicate a yeast/fungal overgrowth of the gastrointestinal (GI) tract. Prescription or natural (botanical) anti-fungals, along with supplementation of high potency multi-strain probiotics, may reduce yeast/fungal levels.

High hippuric acid (10) may derive from food, GI bacterial activity, or exposure to the solvent toluene. Hippuric acid is a conjugate of glycine and benzoic acid formed in the liver. Most hippuric acid in urine is derived from microbial breakdown of chlorogenic acid to benzoic acid. Chlorogenic acid is a common substance in beverages and in many fruits and vegetables, including apples, pears, tea, coffee, sunflower seeds, carrots, blueberries, cherries, potatoes, tomatoes, eggplant, sweet potatoes, and peaches. Benzoic acid is present in high amounts in cranberry juice and is a food preservative. The workplace is the most common source of toluene exposure, but toluene may be absorbed from outgassing of new carpets and other building materials, or absorbed during recreational abuse of solvents such as glue-sniffing. Because most hippuric acid in urine is from GI sources, this marker is a poor indicator of toluene exposure and is being replaced by other markers in occupational safety testing. Bacterial overgrowth can be treated with natural anti-bacterial agents and/or probiotics (30-50 billion cfu's) that include *Lactobacillus rhamnosus*.

High HPHPA (3-(3-hydroxyphenyl)-3-hydroxypropionic acid) (16) is an abnormal phenylalanine metabolite produced when byproducts of *Clostridium* bacteria combine with human metabolites. High concentrations of this compound cause abnormal behavior by inhibiting metabolism of dopamine to epinephrine, resulting in high levels of the dopamine metabolite homovanillic acid (HVA) in the urine and insufficient epinephrine/norepinephrine in the body. It is associated with behavioral, gastrointestinal, and neuropsychiatric symptoms including tic disorders, depression, autism, schizophrenia, aggression, seizures, anorexia, obsessive compulsive disorder, and hyperactivity. Neuropsychiatric effects are more common when values exceed 500 mmol/mol creatinine.

The *Clostridia* species that cause the greatest quantities of urinary HPHPA are *C. sporogenes, C. caloritolerans,* and *C. botulinum*. Additionally, *C. mangenoti, C. ghoni, C. bifermentans, C. caproicum, and C. sordellii* are also capable of causing elevated urinary levels of HPHPA.

HPHPA precursors are not produced by *C.perfringens* -types A-F*, C.tetani, C.subterminale C.capitovale, C.septicum, C.difficile, C.histolyticum,* or *C.tertium.*

C. botulinum would appear to be an unlikely source unless clinical symptoms of botulism are present. The botulinum toxin can cause a severe flaccid paralytic <http://en.wikipedia.org/wiki/Flaccid_paralysis> disease in humans and animals and is the most potent toxin known to humankind, with a lethal dose of less than 1 µg in humans. Symptoms of botulism include weakness, impaired vision, fatigue, and impaired speech. This may then be followed by weakness of the arms, chest muscles and legs. Surprisingly, symptoms may sometimes be mild and the severity of symptoms appears to be modulated by the amount of beneficial flora in the intestinal tract. In food borne botulism, symptoms generally begin 18 to 36 hours after eating contaminated food, but they can occur as early as 6 hours or as late as 10 days. *C. caloritolerans* is so named because it can survive at the boiling point for 8 hours. Its extreme resistance to heat may allow common food borne transmission. *C. sporogenes* is the name given to strains of *Clostridium botulinum* that do not produce botulinum <http://en.wikipedia.org/wiki/Botulinum> neurotoxins. *C. sporogenes* differs from C. botulinum by a single gene. C. sporogenes is ubiquitous in nature and is commonly found in the flora of humans. *C. sordellii* can be pathogenic and has been implicated in fatal toxic shock syndrome among women of child bearing age.

Treatment with Metronidazole or Vancomycin is close to 100% effective at killing parent organisms but not their spores. At least three months of probiotic therapy is recommended after antimicrobial treatment due to spore formation by *Clostridia* species. *Clostridia* overgrowth can sometimes be controlled by supplementation with *Corebiotic*, *Lactobacillus rhamnosus GG* (Culturelle) or *Saccharomyces boulardii.* Phenalalanine or tyrosine supplements should be avoided because of the possibility of conversion to HPHPA or other toxic byproducts.

High 4-cresol (p-cresol) (17) in the urine is most commonly due to *C. difficile* and *C. scatologenes* in the gastrointestinal tract. Most other *Clostridia* species and all other bacteria species do not produce this metabolite. Since *C. scatologenes* is not commonly isolated from stool samples, *C. difficile* would be the most likely source of this compound.

The major clinical significance of 4-cresol is that it is a potent inhibitor of brain dopamine-beta-hydroxylase, the enzyme that converts dopamine to norepinephrine. High concentrations of this compound cause abnormal behavior by inhibiting metabolism of dopamine to norepinephrine, resulting in high levels of the dopamine metabolite homovanillic acid (HVA) in the urine and insufficient norepinephrine in the central nervous system. High urine values of 4-cresol are associated with the most severe clinical symptoms in autism, multiple sclerosis, neurotoxicity, hallucinations, and other neurological and psychiatric disorders. 4-Cresol is also a metabolite of toluene, wood tar creosote, and menthofuran (derived from the mint flavoring agent pennyroyal).

Treatment with Metronidazole or Vancomycin is almost 100% effective in killing parent organisms, including *C. difficile,* but not their spores. At least three months of probiotic therapy is recommended after antimicrobial treatment due to spore formation by *Clostridia* species. *Clostridia* overgrowth can sometimes be controlled by supplementation with *Lactobacillus rhamnosus GG* (Culturelle) or *Saccharomyces boulardi.*

High glyceric (19): may be due to microbial sources such as yeast (Aspergillus, Penicillium, Candida) or due to dietary sources containing glycerol/glycerine.

High glycolic (20): in the absence of oxalic is most likely a result of GI yeast overgrowth (Aspergillus, Penicillium, Candida) or due to dietary sources containing glycerol/glycerine. Glycolic acid had also been found to be a metabolite in Acetobacter, Acidithiobacillus, Alcanligenes, Corynebacterium, Cryptococcus, Escherichia, Gluconobacter, Kluyveromyces, Leptospirillum, Pichia, Rhodococcus, Rhodotorula and Saccharomyces (PMID: 11758919; PMID: 26360870; PMID: 14390024).

High oxalic (21) with or without elevated glyceric (19) or glycolic acids (20) may be associated with the genetic hyperoxalurias, autism, women with vulvar pain, fibromyalgia, and may also be due to high vitamin C intake. However, kidney stone formation from oxalic acid was not correlated with vitamin C intake in a very large study. Besides being present in varying concentrations in most vegetables and fruits, oxalates, the mineral conjugate base forms of oxalic acid, are also byproducts of molds such as *Aspergillus* and *Penicillium* and probably *Candida*. If yeast or fungal markers are elevated, antifungal therapy may reduce excess oxalates. High oxalates may cause anemia that is difficult to treat, skin ulcers, muscles pains, and heart abnormalities. Elevated oxalic acid is also the result of anti-freeze (ethylene glycol) poisoning. Oxalic acid is a toxic metabolite of trichloroacetic acid and other environmental pollutants. In addition, decomposing vitamin C may form oxalates during transport or storage.

Elevated oxalate values with a concomitant increase in glycolic acid may indicate genetic hyperoxaluria (type I), whereas increased glyceric acid may indicate a genetic hyperoxaluria (type II). Elevated oxalic acid with normal levels of glyceric or glycolic metabolites rules out a genetic cause for high oxalate. However, elevated oxalates may be due to a new genetic disorder, hyperoxaluria type III.

Regardless of its source, high oxalic acid may contribute to kidney stones and may also reduce ionized calcium. Oxalic acid absorption from the GI tract may be reduced by calcium citrate supplementation before meals. Vitamin B6, arginine, vitamin E, chondroitin sulfate, taurine, selenium, omega-3 fatty acids and/or N-acetyl glucosamine supplements may also reduce oxalates and/or their toxicity. Excessive fats in the diet may cause elevated oxalate if fatty acids are poorly absorbed because of bile salt deficiency. Unabsorbed free fatty acids bind calcium to form insoluble soaps, reducing calcium's ability to bind oxalate and increase its absorption. If taurine is low in a plasma amino acid profile, supplementation with taurine (1000 mg/day) may help stimulate bile salt production (taurocholic acid), leading to better fatty acid absorption and diminished oxalate absorption.

High levels of oxalates are common in autism. Malabsorption of fat and intestinal *Candida* overgrowth are probably the major causes for elevated oxalates in this disorder. Even individuals with elevated glyceric or glycolic acids may not have a genetic disease. To rule out genetic diseases in those people with abnormally high markers characteristic of the genetic diseases, do the following steps: (1) Follow the nutritional steps indicated in this interpretation for one month; (2) If *Candida* is present, treat *Candida* for at least one month; (3) Repeat the organic acid test after abstaining from vitamin C supplements for 48 hours; (4) If the biochemical markers characteristic of genetic oxalate disorders are still elevated in the repeat test, consider DNA tests for the most common mutations of oxalate metabolism. DNA testing for type I hyperoxaluria is available from the Mayo Clinic, Rochester, MN as test #89915 "*AGXT* Gene, Full Gene Analysis" and, for the p.Gly170Arg mutation only, as # 83643 "Alanine:Glyoxylate Aminotransferase [*AGXT*] Mutation Analysis [G170R], Blood"). Another option to confirm the genetic disease is a plasma oxalate test, also available from the Mayo Clinic (Phone 507.266.5700). Plasma oxalate values greater than 50 micromol/L are consistent with genetic oxalate diseases and may serve as an alternate confirmation test.

Bone tends to be the major repository of excess oxalate in patients with primary hyperoxaluria. Bone oxalate levels are negligible in healthy subjects. Oxalate deposition in the skeleton tends to increase bone resorption and decrease osteoblast activity.

Oxalates may also be deposited in the kidneys, joints, eyes, muscles, blood vessels, brain, and heart and may contribute to muscle pain in fibromyalgia. Oxalate crystal formation in the eyes may be a source of severe eye pain in individuals with autism who may exhibit eye-poking behaviors. High oxalates in the GI tract also may significantly reduce absorption of essential minerals such as calcium, magnesium, zinc, and others. In addition, oxalate deposits in the breast have been associated with breast cancer.

A low oxalate diet may also be particularly useful in the reduction of body oxalates even if dysbiosis of GI flora is the major source of oxalates. Foods especially high in oxalates include spinach, beets, chocolate, soy, peanuts, wheat bran, tea, cashews, pecans, almonds, berries, and many others. A complete list of high oxalate foods is available online at <http://www.greatplainslaboratory.com/home/eng/oxalates.asp>.

People with abnormally high markers characteristic of the genetic diseases should do the following:
1. Avoid spinach, soy, nuts, and berries for one month.
2. If *Candida* is present, treat *Candida* for at least one month.
3. Repeat the organic acid test having abstained from vitamin C supplements for 48 hours.
4. If the biochemical markers characteristic of genetic oxalate disorders are still elevated in the repeat test, consider DNA tests for the most common mutations of oxalate metabolism.

High succinic acid (24) The most common cause of elevated succinic acid is exposure to toxic chemicals which impairs mitochondria function. The most useful tests for confirming toxic chemical exposure are **The Great Plains Laboratory GPL-TOX test** on urine for 172 chemicals and the hair metals test. Succinic acid is metabolized by the mitochondrial enzyme succinic dehydrogenase, which is significant in that it is both a Krebs cycle enzyme and a component- complex 2-of the mitochondrial electron transport chain, making this metabolite a marker of mitochondrial complex 2 as well as Krebs cycle dysfunction. A sampling of toxic chemicals that have been associated with mitochondrial dysfunction include glyphosate, 2, 4-dichlorophenoxyacetic acid (2, 4-D), organophosphate pesticides, mercury, and lead. Approximately 95% of elevated succinic acid results are associated with toxic chemical exposure. Succinic acid in the organic acid test and tiglylglycine in the **GPLTOX test** are two of the most useful markers for mitochondrial dysfunction. Tiglylglycine is a marker for mitochondrial respiratory chain complex I dysfunction while elevated succinic acid indicates respiratory complex 2 dysfunction. Occasionally both succinic acid and tiglylglycine may be elevated in mitochondrial dysfunction. Other Krebs cycle markers may also be elevated when severe chemical toxicity is present. In general, the severity of the chemical toxicity is correlated with higher values of succinic acid.

Less common causes of elevated succinic acid are mitochondrial mutations which may be due to mutations in the nuclear or the mitochondrial DNA for mitochondrial proteins such as Kearns-Sayres disorder. Succinic acid is a metabolite of gamma aminobutyric acid (GABA) so supplementation with GABA may also increase succinic acid.

High fumaric acid (25) may be due to impaired Krebs cycle function, defect of the enzyme fumarase or a defect in mitochondrial function. Recommendations for supporting mitochondrial function include supplementation with coenzyme Q10, L-carnitine or acetyl-L-carnitine, riboflavin, nicotinamide, and vitamin E.* All of these supplements are known to improve mitochondrial dysfunction.

High malic acid (26) indicates a greater requirement for the nutrients niacin and coenzyme Q10.* Malic acid simultaneously elevated with citric, fumaric and alpha-ketoglutaric acids may indicate a possible Cytochrome C Oxidase deficiency. Mitochondrial energy pathway dysfunction would be expected.

High aconitic and citric acids (28, 29) may indicate a need for liposomal glutathione. Glutathione is required for the enzyme aconitase to metabolize citric and aconitic acids. This metabolite may also be associated with mitochondrial energy pathway dysfunction if elevated with other mitochondrial markers such as lactic, pyruvic, malic, fumaric, 3-methylglutaric, 3-hydroxyglutaric, and/or 3-methylglutagonic acids.

High 3-hydroxyglutaric (31) is a metabolite associated with the genetic disease glutaric aciduria type I, which is due to a deficiency of glutaryl CoA dehydrogenase, an enzyme involved in the breakdown of lysine, hydroxylysine, and tryptophan. Other organic acids elevated include glutaric and glutaconic. This disease has been associated with clinical symptoms ranging from near normal to encephalopathy, cerebral palsy, and other neurological abnormalities. Some individuals with glutaric acidemia have developed bleeding in the brain or eyes that may be mistaken for the effects of child abuse . This abnormality should be confirmed by additional testing of enzyme deficiencies and/ or DNA at a major pediatric medical genetics center (Morton et al. Glutaric aciduria type I: a common cause of encephalopathy and spastic paralysis in the Amish of Lancaster County, Pennsylvania. American J. Med. Genetics 41: 89-95, 1991). Elevated values may also be found in hepatic carnitine palmitoyltransferase I deficiency, short-chain acyl dehydrogenase deficiency (SCAD), and ketosis. Mitochondrial dysfunction induced by glutaric acid metabolites causes astrocytes to adopt a proliferative phenotype, which may underlie neuronal loss, white matter abnormalities and macrocephalia. Values in glutaric aciduria type I range from 60-3000 mmol/mol creatinine. Values higher than normal but less than 60 mmol/mol creatinine may be due to mild glutaric acidemia type I or to the other causes indicated above. Treatment of this disorder includes special diets low in lysine and supplementation with carnitine or acetyl-L-carnitine.

High HVA (33) High HVA is usually associated with Clostridia colonization or excess fusaric acid from fungus of the gastrointestinal tract and/or deficiencies of dopamine-beta-hydroxylase (DBH) activity due to single nucleotide polymorphisms (SNPs) or genetic deletions that code for enzymes with low activity. The Great Plains Laboratory now offers a test for the activity of the DBH enzyme on blood serum. The genetic deficiencies of DBH can be treated with the drug Droxidopa (L-*threo*-dihydroxyphenylserine). Droxidopa has the ability to cross the blood brain barrier and be converted to norepinephrine by an alternate biochemical pathway that bypasses the DBH genetic block. Individuals with genetic deficiencies of DBH may have orthostatic hypertension and hypoglycemia and may be more susceptible to attention deficit disorder, Alzheimer's disease, and Parkinson's disease, depression, and bipolar depression. The severity of ADHD symptoms is related to decreased DBH enzyme activity. Cocaine abusers with low-activity DBH SNPs have increased sensitivity to cocaine-induced paranoia and euphoria. The drugs disulfiram and Etamicastat inhibit DBH and the inhibition of alcohol, drug, and gambling addictions by disulfiram may be mediated by DBH inhibition.

If HVA is elevated and VMA is normal and the patient has elevated Clostridia markers, avoid supplementation with L-DOPA, phenylalanine or tyrosine until *Clostridia* is treated. Homovanillic acid (HVA), a dopamine metabolite, is often elevated due to stress-induced catecholamine output from the adrenal gland which depletes vitamin C. Supplementation with vitamin C (ascorbate) may be helpful in such cases.* Elevated HVA can result from the intake of L-DOPA, dopamine, phenylalanine, or tyrosine. Elevated HVA may also result from ingestion of aspartame (Nutrasweet®), salicylates (aspirin), and dietary salicylates. For more information about salicylates in foods go to <http://www.feingold.org/salicylate.php>. Elevated HVA may also result from toxic metal exposure (including lead, aluminum, manganese,arsenic, and mercury), presumably due to DBH inhibition. Heavy metal testing (blood or hair) might be useful to determine if such exposure is significant.

If values are more than double the upper limit of normal, toxoplasmosis and tumors such as neuroblastoma, or other catecholamine-secreting tumors should be ruled out. Catecholamine-secreting tumors can be ruled out by 24- hour VMA and/or HVA testing in urine. Even in this subgroup, the incidence of tumors is extremely rare.

High HVA/VMA ratio (35) the HVA/VMA ratio reflects the balance between dopamine and norepinephrine/epinephrine production by catecholamine producing neurons in the central nervous system, sympathetic nervous system, and adrenal gland. The most common reason for an elevation of the HVA/VMA ratio is a decreased conversion of dopamine to norepinephrine. The enzyme responsible for this conversion, dopamine beta-hydroxylase (DBH), is copper and vitamin C dependent so an elevated ratio could be due to deficiencies of these cofactors. **The most common reason** for this elevated ratio is inhibition of this enzyme by Clostridia byproducts including HPHPA, 4-cresol, or 4-hydroxyphenylacetic acid. Other causes of an increased ratio include inhibition of DBH by the mold metabolite fusaric acid, pharmaceuticals such as disulfiram, or food additives like aspartame. Another cause for an elevated ratio is a genetic variation (single nucleotide polymorphism or SNP) of the DBH enzyme. Alternatively, the activity of the DBH enzyme can be measured on blood serum. Individuals with low DBH activity can be treated with the drug Droxidopa™, which provides adequate norepinephrine by an alternate biochemical pathway. This DBH test on blood serum is now available at The Great Plains Laboratory. High ratios are common in a large number of neuropsychiatric diseases regardless of the reason for DBH deficiency.

High 3,4-dihydroxyphenylacetic acid (DOPAC) (36) 3,4-dihydroxyphenylacetic acid (DOPAC) is an intermediate in the metabolism of dopamine. Values may be elevated due to increased intake of amino acid precursors of DOPAC such as phenylalanine, tyrosine, or DOPA. Values may be elevated due to factors that inhibit dopamine beta hydroxylase (DBH) like Clostridia metabolites, the mold metabolite fusaric acid, pharmaceuticals such as disulfiram, or food additives like aspartame, or to deficiencies of the DBH enzyme due to copper deficiency, vitamin C deficiency, or malic acid deficiency. Single nucleotide polymorphisms (SNPs) of DBH or catechol-O-methyltransferase (COMT) that result in reduced enzyme activities also result in increased amounts of DOPAC. SNPs of COMT are available on **The Great Plains Laboratory DNA methylation pathway test** which can be performed on a cheek swab. Deficiencies of S-adenosylmethionine (S-ame) also are associated with high amounts of DOPAC. DOPAC may also be increased when bananas are ingested the day before urine collection.

High quinolinic acid (39) may be a sign of inflammation and/or neural excitotoxicity. Quinolinic acid is derived from the amino acid tryptophan and is neurotoxic at high levels. As an excitotoxic stimulant of certain brain cells that have NMDA-type receptors, high quinolinic acid may cause nerve cell death with continuous stimulation. Brain toxicity due to quinolinic acid has been implicated in Alzheimer's disease, autism, Huntington's disease, stroke, dementia of old age, depression, HIV-associated dementia, and schizophrenia. High levels of quinolinic acid may inhibit heart contractions, cause lipid peroxidation in the brain, and increase apoptosis (programmed cell death) of astrocytes in human brain. The level of quinolinic acid is also highly correlated with the degree of arthritis impairment.

Quinolinic acid is also a metal chelator, and inhibits enzymes that allow the body to produce glucose when needed. Excessive immune stimulation and chronic inflammation, resulting in overproduction of cytokines like interferon, stimulates overproduction of quinolinic acid. However, quinolinic acid is an important intermediate in making the essential nutritional cofactor nicotinamide adenine dinucleotide (NAD), which is also derived from niacin (B3). Phthalates inhibit the conversion of quinolinic acid to NAD.

Treatment of excessive levels of quinolinic acid can be achieved by multiple approaches: reducing tryptophan supplements, preventing repeated infections and subsequent immune overstimulation by: supplementation with colostrum, transfer factor and probiotics; reducing the use of immune modulators like interferon that increase quinolinic acid production; or reducing the numbers of vaccines given at one time or increasing the interval between vaccinations. The dietary supplements B6 (pyridoxine) and magnesium may reduce brain damage caused by quinolinic acid. A high quinolinic acid/ 5-hydroxyindoleacetic acid ratio would be indicative of immune overstimulation and/or phthalate toxicity.

High uracil (41) can be associated with disorders of folate metabolism, folate deficiency, and genetic disorders of pyrimidine metabolism. Genetic disorders of pyrimidine metabolism are more common when uracil exceeds 50 mmol/mol creatinine and thymine is also elevated. An autistic child with a uracil value >300 mmol/ mol creatinine and diffuse demyelination of the brain was treated with high levels of folate which normalized the uracil but did not improve the clinical symptoms.

High ethylmalonic, methylsuccinic, adipic, suberic, or sebacic acids (45,46,47,48,49) may be due to fatty acid oxidation disorders, carnitine deficiency, fasting, or to increased intake of the medium-chain triglycerides found in coconut oil, MCT oil, and some infant formulas. The fatty acid oxidation defects are associated with hypoglycemia, apnea episodes, lethargy, and coma. [An acyl carnitine profile (Duke University Biochemical Genetics Laboratory, http://medgenetics.pediatrics.duke.edu) can rule out fatty acid oxidation defects.] Regardless of cause, supplementation with L-carnitine or acetyl-L-carnitine may be beneficial.

High pyridoxic acid (51) indicates high recent intake of vitamin B6. Pyridoxic acid is a major metabolite of vitamin B6. Because some individuals may require very high doses of vitamin B6, high values do not necessarily indicate the need to reduce vitamin B6 intake.

High pantothenic acid (B5) (52) most commonly indicates recent intake of pantothenic acid as a supplement. Pantothenic acid is an essential B vitamin that is converted to coenzyme A (unrelated to vitamin A). Coenzyme A is needed for the synthesis of fatty acids, cholesterol, and acetyl choline and is also needed for the Krebs cycle and fatty acid catabolism. Because some individuals may require high doses of pantothenic acid, high values do not necessarily indicate the need to reduce pantothenic acid intake. However, if a patient who **does not take B-vitamin** supplements has high values of pantothenic acid, especially if the values are 20 or more times the upper limit of normal, the individual may have a genetic deficiency in the conversion of pantothenic acid to pantothenic acid-phosphate, which is the first step in the production of coenzyme A. It may be useful to retest after one week off all B-vitamin supplementation; individuals with PKAN would be expected to still have very elevated pantothenic acid levels even with no supplementation. This disease is called pantothenate kinase-associated neurodegeneration (PKAN), an inborn error of metabolism characterized by iron accumulation in the basal ganglia and by the presence of dystonia, dysarthria, Parkinson symptoms, and retinal degeneration. In mild variants of this disease, psychiatric illnesses such as schizoaffective disorder, hallucinations, obsessive compulsive disorder, speech defects, and depression are common. Mutations in pantothenate kinase 2 (PANK2), the rate-limiting enzyme in mitochondrial coenzyme A biosynthesis, represent the most common genetic cause of this disorder. Other biochemical abnormalities commonly found on the organic acid test in this disorder include elevated lactate, pyruvate, and Krebs cycle intermediates. Confirmation of mutant DNA requires special genetic testing. The University of Chicago does testing for PANK2 deletion for a price of $1000 in 2017.
The link is: <http://dnatesting.uchicago.edu/tests/pank2-deletionduplication-analysis>

Treatment for the illness is currently focused on giving high doses of pantothenic acid to stimulate any residual enzyme. Doses as high as 10 g per day have been ingested with few side effects. Other suggested therapies are increased supplementation with cholesterol, fat soluble vitamins, and bile salts. Since Lactobacillus species produce pantothenic acid phosphate, supplementation with high doses of probiotics might also be beneficial.

Ascorbic acid (vitamin C) levels below the mean (54) may indicate a less than optimum level of the antioxidant vitamin C. Individuals who consume large amounts of vitamin C can still have low values if the sample is taken 12 or more hours after intake. Supplementation with buffered vitamin C taken 2 or 3 times a day is suggested.

High 3-hydroxy-3-methylglutaric acid (55) is seen in the genetic disease 3-hydroxy 3-methylglutaric aciduria. Typical values observed in the genetic disease are 200-11,000 mmol/mol creatinine. The cause of less significant increases in this urinary metabolite is unknown. 3-Hydroxy-3-methylglutaric aciduria may cause vomiting, lethargy, hypotonia, and apnea, sometimes evolving to coma. Laboratory tests reveal metabolic acidosis with severe hypoketotic hypoglycemia on fasting or during acute illness, hyperammonemia, and abnormal liver function tests. Preliminary diagnosis is based on a pattern of organic acids in urine which includes 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric, and 3-methylcrotonic acids. Because yeast also produces this compound and yeast metabolites are frequently elevated along with this compound, slight increases may be yeast-related. Reduced activity of 3-hydroxy 3-methylglutaryl Co A reductase, a critical enzyme at the beginning of the cholesterol synthesis pathway, may also elevate this compound. Check cholesterol values when this compound is elevated up to 300 mmol/mol creatinine. Slight elevations may result from coenzyme Q10 deficiency. Supplementation with coenzyme Q10 may be beneficial.

High methylcitric acid (57) is commonly due to biotin deficiency. Biotin is an essential B vitamin. Biotin deficiency may be due to malabsorption, excessive intake of raw egg white, dietary deficiency, or dysbiosis. Methylcitric values greater than 100 mmol/mol creatinine may be due to inborn errors of metabolism involving biotin-dependent enzymes and may require biotin supplementation at very high doses. A high quality multivitamin with biotin or biotin as a single supplement is recommended.

Slightly elevated orotic acid (60) levels less than 1.5 mmol/mol creatinine are commonly associated with dysbiosis. In this case, the use of probiotics may be beneficial.

High 2-hydroxyhippuric acid (61) may result from ingestion of aspartame (Nutrasweet®), salicylates (aspirin), dietary salicylates, or from GI bacteria converting tyrosine or phenylalanine to salicylic acid. For more information about salicylates in foods go to <http://www.feingold.org/salicylate.php>. 2-Hydroxyhippuric acid is a conjugate of hydroxybenzoic acid (salicylic acid) and glycine. Very high 2-hydroxyhippuric also inhibits dopamine beta-hydroxylase resulting in elevated HVA, decreased VMA, and elevated HVA/VMA ratio.

High quality nutritional supplements can be purchased through your practitioner or at New Beginnings Nutritionals, www.NBNUS.com <http://www.NBNUS.com> , or call 877-575-2467.

The nutritional recommendations in this test are not approved by the US FDA. Supplement recommendations are not intended to treat, cure, or prevent any disease and do not take the place of medical advice or treatment from a healthcare professional.