Supplemental Material: Metabolic tables of ISj, meta IMD ™

Table 1. Meta IDM disorders summary for ISj, before Omega 3 therapy. Out of range metabolites for analytically and clinically validated disorders in plasma. An arbitrary threshold (>50% of associated biomarkers) was set to identify disorders potentially associated with the patient sample. Green color represents disorders for which 50% or fewer of the panel metabolites were outside the expected range. Red color represents disorders for which greater than 50% of the panel metabolite Z-scores were outside of the expected range based on the reference cohort. “Metabolites out of Expected Range” denotes the number of panel metabolites out of the expected range relative to the total number of panel metabolites. Molecules were defined as out of the expected range if their Z-score was < -2 or >2 or if present and classified as rare.

Meta IMD™ Disorder	Inside Expected Range	Outside Expected Range	Metabolites Out of Expected Range
2-Hydroxyglutaric Aciduria			0/1
3-Hydroxyisobutyryl-CoA hydrolase (3-HIBCH) Deficiency			0/1
3-Methylcrotonyl-CoA Carboxylase (3MCC) Deficiency			0/5
3-Methylglutaconic Aciduria (MGA)			0/1
4-Aminobutyrate Aminotransferase (ABAT) Deficiency			1/2
Adenylosuccinate Lyase (ADSL) Deficiency			0/1
Argininemia			1/13
Argininosuccinic Acid Lyase (ASL) Deficiency			0/5
Aromatic Amino Acid Decarboxylase (AADC) Deficiency			0/4
Beta-Ureidopropionase Deficiency			0/3
Biotinidase Deficiency			0/3
Carnitine Palmitoyltransferase type II (CPT II) Deficiency			0/4
Citrate Transporter (SLC13A5) Deficiency			0/3
Citrullinemia			0/3
Cobalamin (Cbl) Deficiencies			0/3
Enoyl-CoA hydratase, short chain (ECHS1 or SCEH) Deficiency			0/1
Familial Hypercholanemia			1/2
Gamma-Butyrobetaine Hydroxylase (BBOX) Deficiency			1/11
Glutaric Aciduria type 1 (GA type 1)			0/1
Glutaric Aciduria type 2 (GA type 2)			0/6
Glycerol Kinase Deficiency (GKD)			0/1
HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) Lyase Deficiency			0/7
Holocarboxylase (Multiple Carboxylase) Deficiency			0/4
Homocystinuria			0/4
Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH)			0/5
Hyperphenylalaninemia			0/2
Isovaleric Acidemia			0/7
Lysinuric Protein Intolerance			0/5
Maple Syrup Urine Disease (MSUD)			0/14
Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency			0/6
Mental Retardation, Enteropathy, Deafness, Neuropathy, Ichthyosis, Keratodermia (MEDNIK) Syndrome			0/4
Methylmalonic Acidemia (MMA)			0/7
Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)			0/6
Mitochondrial myopathy, Lactic Acidosis, and Sideroblastic Anemia (MLASA)			0/11
Mucopolysaccharidosis type I (Hurler-Scheie Syndrome)			0/2
Ornithine transcarbamylase deficiency			0/3
Phenylketonuria (PKU)			0/5
Primary Bile Acid Disorders			0/13
Primary Carnitine Deficiency			0/13
Progressive Familial Intrahepatic Cholestasis (PFIC) 2 (Byler's Disease)			0/2

Meta IMD™ Disorder	Inside Expected Range	Outside Expected Range	Metabolites Out of Expected Range
Propionic Acidemia			0/4
Sarcosinemia			0/5
Short Chain Acyl-CoA Decarboxylase (SCAD) Deficiency			0/3
Smith-Lemli-Opitz Syndrome			0/2
Smith-Magenis Syndrome			0/1
Succinic Semialdehyde Dehydrogenase Deficiency			1/3
Tetrahydrobiopterin (THB, BH4) Deficiency			0/6
Thymidine Phosphorylase (MNGIE) Deficiency			0/2
Transaldolase Deficiency			0/6
Trimethyllysine Hydroxylase Epsilon (TMLHE) Deficiency			0/2
Tyrosinemia and Tyrosinemia type 1			0/6
Urocanase Deficiency			0/2
Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) Deficiency			0/9
Zellweger Spectrum Disorder (Peroxisomal Biogenesis Disorder, PEX1 mutations)			0/14
tRNA 5-Methylaminomethyl-2-thiouridylate Methyltransferase (TRMU) Deficiency			0/10

Meta IMD™ INHERITED METABOLIC DISORDERS

Metabolites from Top Affected Disorders

Table 1 did not identify disorders potentially associated with patient sample for which greater than 50% of the panel metabolite Z-scores were outside of the expected range based on the reference cohort.

Laboratory Comments: None

TABLE 2. Significantly altered metabolites (z-score >2 or <-2) possibly related to the subject's phenotype, before Omega 3 therapy.

Biochemical name	Z-score	Super Pathway	Sub Pathway
4-guanidinobutanoate	7.83	Amino Acid	Guanidino and Acetamido Metabolism
N-acetylleucine	3.10	Amino Acid	Leucine, Isoleucine and Valine Metabolism
cholate	2.91	Lipid	Primary Bile Acid Metabolism
gluconate	2.68	Xenobiotics	Food Component/Plant
succinimide	2.17	Xenobiotics	Chemical
1-palmityl-2-arachidonoyl-GPC (O-16:0/20:4)*	2.07	Lipid	Plasmalogen
hyocholate	2.07	Lipid	Secondary Bile Acid Metabolism
caproate (6:0)	-2.05	Lipid	Medium Chain Fatty Acid
sphinganine	-2.05	Lipid	Sphingolipid Synthesis
oleate/vaccenate (18:1)	-2.08	Lipid	Long Chain Fatty Acid
sphinganine-1-phosphate	-2.09	Lipid	Sphingolipid Synthesis
docosadienoate (22:2n6)	-2.10	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
phosphoethanolamine (PE)	-2.12	Lipid	Phospholipid Metabolism
dihomolinolenate (20:3n3 or 3n6)	-2.13	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
taurine	-2.14	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
dihomo-linoleoylcarnitine (C20:2)*	-2.21	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
N-acetylphenylalanine	-2.23	Amino Acid	Phenylalanine Metabolism
pantothenate (Vitamin B5)	-2.26	Cofactors and Vitamins	Pantothenate and CoA Metabolism
3-hydroxydecanoate	-2.27	Lipid	Fatty Acid, Monohydroxy
AMP	-2.29	Nucleotide	Purine Metabolism, Adenine containing
N6-carbamoylthreonyladenosine	-2.30	Nucleotide	Purine Metabolism, Adenine containing
3-hydroxybutyrylcarnitine (2)	-2.32	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
linoleate (18:2n6)	-2.33	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
arachidonate (20:4n6)	-2.36	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
sphingosine	-2.37	Lipid	Sphingosines
6-oxopiperidine-2-carboxylate	-2.38	Amino Acid	Lysine Metabolism
4-hydroxychlorothalonil	-2.39	Xenobiotics	Chemical
dihomolinoleate (20:2n6)	-2.40	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
sulfate*	-2.42	Xenobiotics	Chemical
methionine sulfone	-2.50	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
palmitate (16:0)	-2.59	Lipid	Long Chain Fatty Acid
linolenate (18:3n3 or 3n6)	-2.63	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
octadecanedioate (C18)	-2.66	Lipid	Fatty Acid, Dicarboxylate
aconitate [cis or trans]	-2.71	Energy	TCA Cycle
phenyllactate (PLA)	-2.78	Amino Acid	Phenylalanine Metabolism
hexadecadienoate (16:2n6)	-2.89	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
N-acetylglutamate	-2.95	Amino Acid	Glutamate Metabolism
C-glycosyltryptophan	-3.42	Amino Acid	Tryptophan Metabolism
N-acetyl-aspartyl-glutamate (NAAG)	-3.60	Amino Acid	Glutamate Metabolism
beta-alanine	-5.16	Nucleotide	Pyrimidine Metabolism, Uracil containing

Table 3. Meta IDM disorders summary for ISj, after Omega 3 therapy. Out of range metabolites for analytically and clinically validated disorders in plasma. An arbitrary threshold (>50% of associated biomarkers) was set to identify disorders potentially associated with the patient sample. Green color represents disorders for which 50% or fewer of the panel metabolites were outside the expected range. Red color represents disorders for which greater than 50% of the panel metabolite Z-scores were outside of the expected range based on the reference cohort. "Metabolites out of Expected Range" denotes the number of panel metabolites out of the expected range relative to the total number of panel metabolites. Molecules were defined as out of the expected range if their Z-score was < -2 or >2 or if present and classified as rare.

Meta IMD™ Disorder	Inside Expected Range	Outside Expected Range	Metabolites Out of Expected Range
2-Hydroxyglutaric Aciduria			0/1
3-Hydroxyisobutyryl-CoA hydrolase (3-HIBCH) Deficiency			0/1
3-Methylcrotonyl-CoA Carboxylase (3MCC) Deficiency			0/5
3-Methylglutaconic Aciduria (MGA)			0/1
4-Aminobutyrate Aminotransferase (ABAT) Deficiency			0/2
Adenylosuccinate Lyase (ADSL) Deficiency			0/1
Argininemia			0/13
Argininosuccinic Acid Lyase (ASL) Deficiency			0/5
Aromatic Amino Acid Decarboxylase (AADC) Deficiency			0/4
Beta-Ureidopropionase Deficiency			0/3
Biotinidase Deficiency			0/3
Carnitine Palmitoyltransferase type II (CPT II) Deficiency			0/4
Citrate Transporter (SLC13A5) Deficiency			0/3
Citrullinemia			0/3
Cobalamin (Cbl) Deficiencies			0/3
Enoyl-CoA hydratase, short chain (ECHS1 or SCEH) Deficiency			0/1
Familial Hypercholanemia			1/2
Gamma-Butyrobetaine Hydroxylase (BBOX) Deficiency			0/11
Glutaric Aciduria type 1 (GA type 1)			0/1
Glutaric Aciduria type 2 (GA type 2)			0/6
Glycerol Kinase Deficiency (GKD)			0/1
HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) Lyase Deficiency			0/7
Holocarboxylase (Multiple Carboxylase) Deficiency			0/4
Homocystinuria			0/4
Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH)			0/5
Hyperphenylalaninemia			0/2
Isovaleric Acidemia			1/7
Lysinuric Protein Intolerance			0/5
Maple Syrup Urine Disease (MSUD)			0/14
Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency			0/6
Mental Retardation, Enteropathy, Deafness, Neuropathy, Ichthyosis, Keratodermia (MEDNIK) Syndrome			0/4
Methylmalonic Acidemia (MMA)			0/7
Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)			0/6
Mitochondrial myopathy, Lactic Acidosis, and Sideroblastic Anemia (MLASA)			0/11
Mucopolysaccharidosis type I (Hurler-Scheie Syndrome)			0/2
Ornithine transcarbamylase deficiency			0/3
Phenylketonuria (PKU)			0/5
Primary Bile Acid Disorders			0/13
Primary Carnitine Deficiency			0/13
Progressive Familial Intrahepatic Cholestasis (PFIC) 2 (Byler's Disease)			0/2
Propionic Acidemia			0/4

Meta IMD™ Disorder	Inside Expected Range	Outside Expected Range	Metabolites Out of Expected Range
Sarcosinemia			0/5
Short Chain Acyl-CoA Decarboxylase (SCAD) Deficiency			0/3
Smith-Lemli-Opitz Syndrome			0/2
Smith-Magenis Syndrome			0/1
Succinic Semialdehyde Dehydrogenase Deficiency			0/3
Tetrahydrobiopterin (THB, BH4) Deficiency			0/6
Thymidine Phosphorylase (MNGIE) Deficiency			0/2
Transaldolase Deficiency			0/6
Trimethyllysine Hydroxylase Epsilon (TMLHE) Deficiency			0/2
Tyrosinemia and Tyrosinemia type 1			0/6
Urocanase Deficiency			0/2
Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) Deficiency			1/9
Zellweger Spectrum Disorder (Peroxisomal Biogenesis Disorder, PEX1 mutations)			0/14
tRNA 5-Methylaminomethyl-2-thiouridylate Methyltransferase (TRMU) Deficiency			0/10

TABLE 4. Significantly altered metabolites (z-score >2 or <-2) possibly related to the subject's phenotype, after Omega 3 therapy.

Biochemical name	Z-score	Super Pathway	Sub Pathway
1-eicosapentaenoyl-GPC (20:5)*	5.23	Lipid	Lysophospholipid
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	4.17	Lipid	Fatty Acid, Dicarboxylate
1-palmitoyl-2-eicosapentaenoyl-GPC (16:0/20:5)*	3.48	Lipid	Phosphatidylcholine (PC)
eicosapentaenoate (EPA; 20:5n3)	3.47	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
cholate	3.34	Lipid	Primary Bile Acid Metabolism
1-docosapentaenoyl-GPC* (22:5n3)*	2.49	Lipid	Lysophospholipid
alpha-tocopherol	2.32	Cofactors and Vitamins	Tocopherol Metabolism
1-(1-enyl-stearoyl)-2-docosahexaenoyl-GPC (P-18:0/22:6)*	2.19	Lipid	Plasmalogen
1-pentadecanoyl-2-docosahexaenoyl-GPC (15:0/22:6)*	2.15	Lipid	Phosphatidylcholine (PC)
lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	2.11	Lipid	Lactosylceramides (LCER)
carotene diol (1)	2.09	Cofactors and Vitamins	Vitamin A Metabolism
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	2.05	Lipid	Plasmalogen
phosphatidylcholine (16:0/22:5n3, 18:1/20:4)*	2.02	Lipid	Phosphatidylcholine (PC)
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	-2.01	Lipid	Phosphatidylserine (PS)
dihomolinolenate (20:3n3 or 3n6)	-2.04	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
eicosenoylcarnitine (C20:1)*	-2.04	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
N-acetyl-aspartyl-glutamate (NAAG)	-2.05	Amino Acid	Glutamate Metabolism
sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)*	-2.06	Lipid	Sphingomyelins
4-oxo-retinoic acid	-2.07	Cofactors and Vitamins	Vitamin A Metabolism
sphinganine	-2.10	Lipid	Sphingolipid Synthesis
N4-acetylcytidine	-2.15	Nucleotide	Pyrimidine Metabolism, Cytidine containing
margarate (17:0)	-2.16	Lipid	Long Chain Fatty Acid
5-dodecenoate (12:1n7)	-2.17	Lipid	Medium Chain Fatty Acid
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	-2.20	Lipid	Phosphatidylethanolamine (PE)
1-stearoyl-2-docosapentaenoyl-GPE (18:0/22:5n6)*	-2.20	Lipid	Phosphatidylethanolamine (PE)
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	-2.21	Lipid	Phosphatidylethanolamine (PE)
3-hydroxy-3-methylglutarate	-2.22	Lipid	Mevalonate Metabolism
linoleate (18:2n6)	-2.22	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
linolenate (18:3n3 or 3n6)	-2.22	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
adrenate (22:4n6)	-2.24	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	-2.28	Lipid	Phosphatidylethanolamine (PE)
dodecanedioate (C12)	-2.29	Lipid	Fatty Acid, Dicarboxylate
eicosenoate (20:1n9 or 1n11)	-2.30	Lipid	Long Chain Fatty Acid
AMP	-2.31	Nucleotide	Purine Metabolism, Adenine containing
creatine	-2.34	Amino Acid	Creatine Metabolism
1-(1-enyl-stearoyl)-2-dihomo-linolenoyl-GPE (P-18:0/20:3)*	-2.36	Lipid	Plasmalogen
1-stearoyl-2-docosapentaenoyl-GPC (18:0/22:5n6)*	-2.36	Lipid	Phosphatidylcholine (PC)
pantothenate (Vitamin B5)	-2.36	Cofactors and Vitamins	Pantothenate and CoA Metabolism
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	-2.37	Lipid	Phosphatidylethanolamine (PE)
lactate	-2.39	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
linoleoyl ethanolamide	-2.42	Lipid	Endocannabinoid
4-hydroxychlorothalonil	-2.59	Xenobiotics	Chemical
quinolinate	-2.59	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
dihomo-linoleoylcarnitine (C20:2)*	-2.61	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	-2.65	Lipid	Phosphatidylethanolamine (PE)
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	-2.65	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
serotonin	-2.87	Amino Acid	Tryptophan Metabolism
1-stearoyl-2-adrenoyl-GPC (18:0/22:4)*	-2.93	Lipid	Phosphatidylcholine (PC)
1-dihomo-linolenoyl-GPE (20:3n3 or 6)*	-3.00	Lipid	Lysophospholipid
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	-3.00	Lipid	Phosphatidylethanolamine (PE)
dihomolinoleate (20:2n6)	-3.02	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
docosadienoate (22:2n6)	-3.14	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)*	-3.23	Lipid	Phosphatidylethanolamine (PE)
1-palmitoyl-2-adrenoyl-GPC (16:0/22:4)*	-3.48	Lipid	Phosphatidylcholine (PC)

ADDITONAL INFORMATION ABOUT THE Meta IMD™ INHERITED METABOLIC DISORDERS TEST

Meta IMD™ Analytical Methods

Meta IMD™ identifies small molecules (between 50-1,500 Daltons (Da) in molecular weight) in patient samples. This identification is performed using four different types of high-performance Ultra Performance Liquid Chromatography (UPLC) instruments paired with Mass Spectrometry (UPLC/ MS).[1,2] The identification of each molecule is confirmed against a proprietary chemical library consisting of accurate molecular weight/mass plus information on any adductation, in source fragmentation, and/or polymerization (typically dimers and trimers), retention time/index on the chromatography columns, and mass spectral fragmentation patterns.[3] Overall process variability is assessed using stable isotope standards to monitor the performance of the assay and for quality control purposes.

Intended Use

Meta IMD™ analysis of plasma may identify molecules that are reflective of metabolic disease states and secondary effects induced by the disease. This is NOT a stand-alone test for inherited metabolic disorders. In making the clinical diagnosis, the clinician should not rely on Meta IMD™ alone, but rather should consider all analytical results, and the patient's history, signs, and symptoms.

As an adjunctive, first-line clinical test, Meta IMD™ may be considered in the following situations:

1. Individuals with an undifferentiated phenotype suspected to be related to perturbation in a biochemical pathway (e.g. child with developmental delay, seizures, autism, etc.). In these cases, the test:
   - May help to substantiate results from traditional methods such as targeted biochemical analyses and exome sequencing.
   - May help to clarify equivocal results that were produced as a result of routine analytical testing (i.e. discrepant targeted biochemical analysis, genetics variant of unknown significance, etc.).
   - May be used to screen for biochemical perturbations in patient samples. In this case, the test results can be used to assist the clinician in choosing the appropriate diagnostic and confirmatory tests.
   - May be used in cases where routine testing is negative and additional biochemical analysis is required. In this case, the test results may identify abnormalities that were not identified using the routine methods. Under these circumstances, the Meta IMD™ results only are used to guide further diagnostic and confirmatory testing, not to provide a definitive diagnosis.
2. Individuals with equivocal molecular test results in a gene known to be involved in small molecule metabolism.

[1] Evans AM et al. Anal Chem. 2009;81(16):6656-67 doi: 10.1021/ac901536h PMID:19624122

[2] Evans AM et al. Metabolomics. 2014;4(132) doi: 10.4172/2153-0769.1000132

[3] Dehaven CD et al. J Cheminform. 2010;2(1):9 doi: 10.1186/1758-2946-2-9 PMCID:PMC2984397

Reporting of Results

General Information:

- The UPLC/MS signal intensity of each biochemical in the panel is compared to the signal intensities of the biochemical in a reference cohort to calculate a Z-score. The Z-score is a statistical measurement of a score's relationship to the mean in a group of scores and is representative of the standard deviations away from the mean of the reference cohort. The Z-Score calculation *(Xi-meanref)/SDref)* has cut-off points based on the theoretical quintiles of the normal distribution.
- In Meta IMD™, the Z-score cut-off points for being out of range are >2 or <-2. These values are based on the quintiles of the normal distribution, respectively approximating the upper 2.5% and lower 2.5% of the distribution.
- The reference cohort consists of 866 pediatric patient samples. For construction of the reference cohort, molecules in the top 5% and bottom 5% of Z-scores for each individual sample were excluded from the reference population database.

In the Meta IMD™ report:

- A compound is reported if it has a Z-score outside the expected range.
- Individual Z-scores should not be used to draw conclusions about a specific abnormality or inherited metabolic disease state. The Z-scores must be interpreted in the context of the entire metabolic pathway.
- Inherited metabolic disorders are called out if more than 50% of the Z-scores for biomarkers associated with that disorder, including rare biomarkers, are outside the expected Z-score range. For disorders where rare compounds are biomarkers associated with that disorder, they are considered out of range if detected in the sample.
- All Meta IMD™ results must be interpreted in conjunction with all other clinical test results and clinical information about the patient.

Limitations

The following limitations of the test should be understood when analyzing the test data:

- Clinical validation of Meta IMD™ was performed in some, but not all of the clinical uses outlined above. In the cases of confirmed inherited metabolic disorders (IMDs), clinical validation was performed, in a blinded fashion, on small disease cohorts in accordance to the rare disease guidelines provided under Section D.5.(a) draft guidance by the FDA for Laboratory Developed Tests (LDTs) Used for Rare Diseases. These cohorts have at least one confirmed clinical case and at least one associated analytically validated molecule.
- Disorders defined as "out of expected range" in the Meta IMD™ Disorders Summary (**Table 1**) were based on a threshold that has not been validated.
- Failure to detect a compound is not evidence of its absence in the sample.
- Failure to identify a particular small molecule may occur for a number of reasons including: (i) low concentration, (ii) interference from other compounds in the sample masking identification, or (iii) the requirement for special extraction or chromatographic methods.
- Some biochemicals are not typically present at detectable levels in samples from normal/healthy individuals. Since these compounds are very sparse or absent in healthy controls, the calculation of a relative increase (e.g. >2 Z-score) in a patient sample compared to the reference control cohort is not reliable; however, the presence of these molecules in patient samples can be indicative of a metabolic abnormality. Compounds present in less than 10% of the reference population are reported as "rare" in the *Technical QC Document* but not given a Z-score and are considered to be out of range when detected.

- Reporting of disorders in the Meta IMD™ Disorders Summary (**Table 1**) and the ability to Z-score compounds is affected by the detection of a compound in both the patient sample as well as the technical replicate normalizing matrix samples used to scale data for comparison to the reference population. Compounds and disorders listed in **Table 1** that are affected by not being detected in the patient sample or the normalizing matrix technical replicate samples are reported in the *Technical QC Document*.

- All molecules listed in this report are contained in Metabolon's proprietary chemical library and, with the exception of those with an asterisk (*), have been verified with authentic reference standards. In multiple validation studies, Metabolon has verified the analytical performance of 258 molecules in plasma. Other molecules are chemically/structurally similar to analytically validated compounds and consequently are expected to behave in like fashion. The identification of compounds marked with an asterisk (*) is based on mass spectrometry data but no reference standards are currently available to verify the identity.
- Based on our best knowledge, some reported molecules have unknown or non-specific clinical significance.
- While quality control samples and internal reference standards are included in each analytical batch to identify unacceptable instrument performance and to monitor overall process variation, the QC may not detect all sources of variability in this global analysis such as matrix effect and carryover.
- Hemolysis (red blood cell lysis), high lipid content, and/or high protein levels in plasma samples can interfere with the identification and relative quantitation of several biochemicals. Sample collection, processing, and shipping instructions should be carefully followed to avoid hemolysis. Plasma samples with high levels of hemolysis will be reported in the "Laboratory Comments" section above.
- Medications, both over-the-counter and prescription, as well as nutritional interventions, affect the levels of several metabolites and biochemical pathways. These interventions should be noted and considered when analyzing the data.
- Dietary status affects the biochemical levels of several metabolites and activities of several metabolic pathways. The non-fasted/fasted status of an individual should be considered when analyzing the results.

Metabolon Supporting Publications

- Miller, M.J. et al. Untargeted Metabolomic Analysis for the Clinical Screening of Inborn Errors of Metabolism. J Inherit. Metab Dis, 2015 http://www.metabolon.com/resources/publications/list-of-current-publications/april-2015/p0000466.aspx

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