Newborn Screening Unit

Testing Protocols and Cutoff Values

Amino Acids Disorders

Newborn screening dried blood spot (DBS) specimens are analyzed for amino acids by electrospray tandem mass spectrometry. Amino acids are eluted from the dried blood spots, mixed with deuterated internal standards, and then injected into the mass spectrometer. The amino acids included in the analysis are: arginine, citrulline, leucine, methionine, phenylalanine, tyrosine, and valine. Results are reported as µmoles/liter. Specimens with one or more amino acids greater than the cutoff values are reanalyzed in duplicate to confirm that the amino acid concentration is elevated. Cutoff values are listed in Table 1. Also listed are critical values and, when applicable, the secondary markers. Result interpretations depend on whether the concentration of the primary marker is greater than the cutoff or critical value and whether the concentration of the secondary marker is elevated.

Organic Acidemias and Fatty Acid ß-Oxidation Defects

DBS specimens are analyzed for acylcarnitines by electrospray tandem mass spectrometry. This is done in conjunction with the analysis for amino acids. Acylcarnitines are eluted from the dried blood spots, mixed with deuterated internal standards, and then injected into the mass spectrometer. The acylcarnitines along with their cutoff values are listed in Table 1. Results are reported as µmoles/liter. Specimens with one or more acylcarnitines outside of the reference range are reanalyzed in duplicate to confirm that the acylcarnitine concentration is elevated or low. Also listed in Table 1 are critical values and, when applicable, the secondary markers. Result interpretations depend on whether the concentration of the primary marker is greater than the cutoff or critical value and whether the concentration of the secondary marker is elevated.

Galactosemia

DBS specimens are analyzed for activity of the enzyme galactose-1-phosphate uridyltransferase (GALT) using a semi-quantitative fluorescence assay from Astoria Pacific. Specimens exhibiting low or no activity are retested in duplicate and analyzed for total galactose. Specimens confirmed to have low GALT activity and/or elevated total galactose are reported as presumptive positive for galactosemia, and the Emory Department of Human Genetics is notified promptly. The cutoff value for total galactose is 11 mg/dl.

Biotinidase Deficiency

DBS specimens are analyzed for activity of the enzyme biotinidase using a semi-quantitative colorimetric assay from Astoria-Pacific. Specimens exhibiting low or no activity are retested in duplicate. Specimens confirmed to have activity less than the cutoff of 10 ERU are reported as presumptive positive for biotinidase deficiency, and the Emory Department of Human Genetics is notified the same day.

Congenital Hypothyroidism

DBS specimens are analyzed for total thyroxine (T4) and thyroid-stimulating hormone (TSH) using time-resolved fluoroimmunoassays from Perkin Elmer Health Sciences. Specimens exhibiting low levels of T4 or elevated levels of TSH are retested in duplicate. Specimens confirmed to have low T4 or elevated TSH are reported as presumptive positive for congenital hypothyroidism, and the Emory Department of Human Genetics is notified the same day. Cutoff values for T4 and TSH are birth weight and age dependent:

Birth Weight	Age	T4 Cutoff	TSH Cutoff
<2500 g	<24 hr	5.0 µg/dl	50 µIU/ml
<2500 g	>=24 hr	5.0 µg/dl	25 µIU/ml
>=2500 g	<24 hr	8.0 µg/dl	50 µIU/ml
>=2500 g	24 hr – 7 days	8.0 µg/dl	25 µIU/ml
>=2500 g	>=7 days	6.0 µg/dl	25 µIU/ml

Congenital Adrenal Hyperplasia (CAH)

DBS specimens are analyzed for 17α-hydroxy-progesterone (17-OHP) using a time-resolved fluoroimmunoassay from Perkin Elmer Health Sciences. Specimens exhibiting elevated levels of 17-OHP are retested in duplicate. Specimens confirmed to have elevated 17-OHP are reported as presumptive positive for CAH, and the Emory Department of Human Genetics is notified the same day. Cutoff values for 17-OHP are age and weight dependent. A cutoff value of 88 ng/dml serum is used for specimens collected prior to 24 hours of age or from infants weighing less than 2500 grams. A value of 33 ng/dml serum is used for all other specimens.

Hemoglobinopathies

A two-tiered approach is used to analyze DBS specimens for hemoglobin (Hb) variants. The specimens are initially screened by high pressure liquid chromatography (HPLC) using the Variant Hemoglobin Testing System from Bio-Rad Laboratories. All specimens exhibiting a phenotype other than FA or AF are then analyzed by gel isoelectric focusing (IEF).Hemoglobin variants identified include F (fetal), A (adult), S (sickle), C, D, E, and Barts. Most of the time there is agreement between the two methods, and the report gives the Hb variants identified. If there is discordance between the IEF and HPLC results, professional judgment is used in deciding how to report the results. Variants that cannot be positively identified are designated on the report as ‘V’. Phenotypes corresponding to a high probability of a disease state (e.g., FS, FSC) are reported promptly to the Georgia Health Sciences University, Department of Pediatrics or the Georgia Department of Public Health, Newborn Screening Program.

Cystic Fibrosis

A two-tiered approach is used to analyze DBS specimens for cystic fibrosis (CF). The specimens are initially screened for immunoreactive trypsin (IRT) using a time-resolved fluoroimmunoassay from Perkin Elmer Health Sciences. Specimens with an IRT concentration greater than or equal to 55 ng/ml blood are then analyzed for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene using a kit from Hologic, Inc. that tests for

23 of the most common CFTR mutations, as recommended by the American College of Medical Genetics. If one or two mutations are identified, they are reported along with the elevated IRT value. If no mutations are identified, the elevated IRT value is reported along with a comment that no mutations were identified. If the IRT concentration is greater than 128 ng/ml blood and no mutations are identified, the specimen is sent to the Emory Genetics Laboratory (EGL) for analysis for 16 additional rare CFTR mutations. A final report is delayed by 1-2 weeks for specimens sent to EGL.

Cutoff Values

Table 1. Cutoff values (µmoles/liter) for amino acids and acylcarnitines.

Primary Marker	**Cutoff**	**Critical**	**Secondary Marker**	**Cutoff**	**Critical**
ARG (BW ≥2500 grams)	105	140			
ARG (BW <2500 grams)	120	210			
CIT (Age = 0-7 days)	56	85	CIT/ARG	6.0	
CIT (Age >7 days)	80	160	CIT/ARG	6.0	
LEU	350	450	LEU/PHE	4.1	
			VAL	250	
MET (BW ≥2500 grams)	70	150	MET/PHE	1.0	
MET (BW <2500 grams)	150	217	MET/PHE	3.4	
PHE	160	210	PHE/TYR	1.40	
TYR	450	550			
C0-LOW	7.0	4.50			
C3	6.2	8.5	C3/C2[1]	0.22	0.27
C3DC/C4OH	0.50	0.75	C3DC/C10	4.0	
C4	1.30	2.10	C4/C3	1.00	
C5 (BW ≥2500 grams)	0.54	1.00	C5/C3	0.48	
C5 (BW <2500	1.2	1.48	C5/C3	1.00	

grams)					
C5:1	0.25	0.46	C5-OH[2]	0.70	
C5-OH	0.70	0.92	C5-OH/C8	8.0	
C5DC	0.63	0.68	C5DC/C8	3.65	
C8	0.35	0.67	C8/C2[2]	0.025	
C14:1 (Age = 0-7 days)	0.51	0.67	Multiple[3]		
C14:1 (Age >7 days)	0.34	0.67	Multiple[3]		
C16 (Age = 0-7 days)	7.0	9.0	C18:1[1]	2.50	3.57
C16 (Age >7 days)	5.6	6.8	C18:1[1]	2.50	3.57
C16-OH	0.08	0.20	C18:1-OH[4]	0.08	0.20
C0/(C16+C18) (0-7, ≥2500)	32	48	C16 - LOW	1.30	
C0/(C16+C18) (0-7, <2500)	63	94	C16 - LOW	0.43	
C0/(C16+C18) (>7)	100	131	C16 - LOW	0.22	

[1]Both the primary and secondary markers must be elevated, or either must be greater than its critical value.

[2]Secondary marker must be elevated, unless primary marker is greater than the critical value.

[3]There are multiple secondary analytes associated with C14:1.

[4]Both the primary and secondary markers must be elevated.