

Tandem Mass Spectrometry in Newborn Screening

A Primer for Neonatal and Perinatal Nurses

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Since 1961, newborn screening for errors of metabolism (EM) has improved the diagnosis, treatment and outcome of newborns with an EM. Recently, advances in laboratory technology with tandem mass spectrometry (MS/MS) has increased the identification of newborns with an EM. With a single dried filter paper blood spot (Guthrie R, Susi A. A simple phenylalanine method for detecting PKU in large populations of newborn infants. *Pediatrics*. 1963;32:338-343), MS/MS can identify more than 30 disorders of metabolism. This review will explore MS/MS to provide a better understanding of the development and application of this technology to newborn screening for perinatal and neonatal nurses. **Key words:** *errors of metabolism, metabolic diseases, newborn screening, tandem mass spectrometry*

IN 1961, Dr Robert Guthrie developed a sensitive, but simple and inexpensive, screening test for phenylketonuria (PKU).¹ This screening test used a bacterial inhibition assay of dried blood collected on filter paper to measure phenylalanine. With early identification of newborns with PKU, earlier initia-

tion of a diet low in phenylalanine resulted in normal cognitive development, rather than severe mental retardation associated with classical PKU.² By 1962, Oregon and Massachusetts began screening every newborn in those states for PKU.³ By the late 1960s, the practice of routine newborn screening for PKU had expanded to almost every state and soon afterward to most developed countries. Within 2 decades, all states had instituted universal newborn screening for PKU.⁴⁻⁷ Subsequently, newborn screening programs have added screening tests for other disorders, including amino acidopathies, galactosemia, congenital hypothyroidism, and sickle cell disease. The disorders were added based upon principles of newborn screening. Before a disease is considered suitable for addition to the newborn screening test, it should meet the following criteria proposed by Wilson and Jungner:⁸

1. It should be frequent and severe enough to be a public health concern.
2. It should cause a documented spectrum of symptoms.
3. The screening test must be reliable, simple, and have a low incidence of false-positive and false-negative results.

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4. The disease should be treatable to a degree to decrease the significant negative outcomes, such as death.
5. The appropriate confirmatory diagnostic tests must be readily available.
6. There must be an established follow-up program.
7. There should be a positive cost-benefit ratio to society.

These principles emphasize the goals of newborn screening.⁸⁻¹⁰ Few tests and conditions, however, will fulfill all of these criteria and the effect of treatment may not be known for years after a test is introduced. Almost all of the disorders screened and identified by newborn screening programs are Mendelian conditions. However, increasingly other conditions, such as HIV and congenital toxoplasmosis, have been included in some screening programs.^{11,12} For a few of the disorders, the evidence for efficacy of early medical intervention that early screening allowed was overwhelming, as with PKU and congenital hypothyroidism.^{2,13} In other cases, the benefits of medical intervention are less dramatic, but are significant enough to warrant screening; an example is galactosemia. Early identification of newborns with classical galactosemia prevents the early death from hepatocellular damage and gram-negative sepsis.¹⁴ However, a survey of 350 cases revealed that most patients with classical galactosemia, even if diagnosed early and treated, have lower IQ than expected, speech and motor dysfunction, and growth and ovarian failure.¹⁵ The screening prevented mortality, but not the long-term sequelae.

As more disorders were added to the original screening for PKU, the laboratory needs increased as each disorder had a separate bacterial assay. Screening needed to be more efficient and comprehensive. The development of a single assay system that could be used to detect several disorders rather than a system of separate bacterial assays for each disorder was desired. The use of chromatography, which provides analysis by producing bands of color at different levels on a column, rather than bacterial assays was tried.^{16,17} Pa-

per chromatography was too sensitive for utilizing to screen newborn blood within the first days of life when the majority of specimens are collected. Next, thin-layer chromatography was explored for screening newborn urine.¹⁸ This form of chromatography had several disadvantages.¹⁸ First, the urine specimen could not replace blood in detecting either PKU or congenital hypothyroidism. The second issue was the collection of urine specimens after discharge from the nursery—a logistic nightmare for the parents and the healthcare providers. Third, the concentration of urine varies from being highly concentrated and producing false-positive results to highly dilute and causing false-negative results. Lastly, several of the disorders identified by urine analysis are benign, such as histidinemia and Hartnup disorder. As a result, newborn urine screening based upon chromatography has been discontinued.¹⁹ Universal newborn screening continued to rely on the “one test-one disorder” system. With advances in technology, tandem mass spectrometry (known as *MS/MS*) has changed universal newborn screening.^{20,21} The use of *MS/MS* allows for screening of multiple metabolic disorders using a single analytical run. With this technology, a numerous array of metabolic disorders, including amino acids disorders, fatty acid oxidation disorders, and organic acidemias have been identified during the neonatal period many times prior to a catastrophic event (Table 1).²⁵⁻²⁹ The purpose of this review on *MS/MS* is to provide a better understanding of this powerful technology and why it is important for nurses to understand.

MASS SPECTROMETER

A tandem mass spectrometer is a type of analytical instrument used in laboratories known as a *mass spectrometer* (Fig 1).³⁰ The mass spectrometer instrument was developed more than 20 years ago.^{20,31} This instrument can analyze numerous compounds, such as those in body fluids, environmental

Table 1. Summary of disorders detectable through tandem mass spectrometry during the neonatal period.*

Amino acid disorders	Fatty acid disorders	Organic acid disorders
Argininemia	Carnitine/acylcarnitine	2-methylbutyryl-
Argininosuccinic Acid (ASA) lyase deficiency)	translocase deficiency (CAT)	CoAdehydrogenase deficiency (2MBCD or SBCAD)
Citrullinemia (ASA synthetase deficiency)	Carnitine palmitoyl transferease deficiency, type I (CPT-I)	2-methyl-3-OH butyric aciduria
Homocystinuria (HCU)	Carnitine palmitoyl transferase deficiency, type II (CPT-II)	3-methylcrotonyl-CoA carboxylase deficiency (3MCC)
Hyperphenylalaninemias (PKU, HPhe)	Carnitine transport defect	3-methylgluta-conyl-CoA hydratase deficiency
Classical PKU, Hyperphenylalaninemia, Bipterin cofactor defects	Long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency/Trifunctional protein deficiency (TPF)	3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG)
Maple syrup disease (MSD)	Medium chain acyl-CoA dehydrogenase (MCAD) deficiency	Glutaric acidemia, type I (GA-I)
	Multiple acyl-CoA dehydrogenase deficiency (MADD or GAIL, Glutaric acidemia, type II)	Isovaleric acidemia (IVA)
	Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency	Malonic acidemia
		Methylmalonic acidemias (MMA)
		Mitochondrial acetoacetyl-CoA thiolase deficiency (SKAT, BKT, 3-ketothiolase deficiency, β ketothiolase)
		Multiple carboxylase deficiency (MCD)
		Propionic acidemia (PA)

*Adapted from Millington and Koeberl,²² Zytkevich et al,²³ and Rashed et al.²⁴

contaminates of water, foods, and pharmaceuticals. Simplistically, the mass spectrometer weighs molecules (Fig 2). Molecules are extremely small and cannot be weighed in the usual method on a scale. The mass spectrometer weighs molecules electronically. Every molecule has a unique mass. For example, the mass of water is 18. In addition to identifying a compound by its mass, the mass spectrometer determines how much of the compound is present in the material that is being analyzed. The best analogy for this is pocket change. When you grab a handful of coins, you may have pennies, dimes, nickels, and quarters. If you sort these from lightest to heaviest, the dimes would be the lightest while the quarters would be the heaviest. The other coins would fall between the dimes and the quarters. The mass spectrometer sorts molecules in much the same method.

TANDEM MASS SPECTROMETER

The tandem mass spectrometer was further refined a decade ago for clinical use by researchers at Duke University in collaboration with the North Carolina Health Department.^{20,21,32} A tandem mass spectrometer is simply 2 mass spectrometers hooked together with a special chamber between the 2 instruments (Fig 3).³³ Thus, the abbreviation of MS/MS for tandem mass spectrometry (MS - chamber - MS = MS/MS). After being prepped, the sample is injected into the first instrument. While in the first instrument, the sample is ionized to produce molecular ions and the type of molecules present are determined based upon mass-to-charge (m/z) ratio. The ionized molecules are sorted and weighed. Afterward, the sample is sent into the collision cell chamber. Within the



Figure 1. Tandem mass spectrometer. Permission from Waters Corporation (MS Technologies Centre).

collision cell chamber, the molecular ion sample is broken into fragmented pieces, called *analytes*, which are like pieces of a puzzle. After being fragmented, the sample

is passed into the second instrument. Within the second instrument, quantities of the selected analyte(s) are sorted and weighed according to their *m/z* ratio. The peak of each

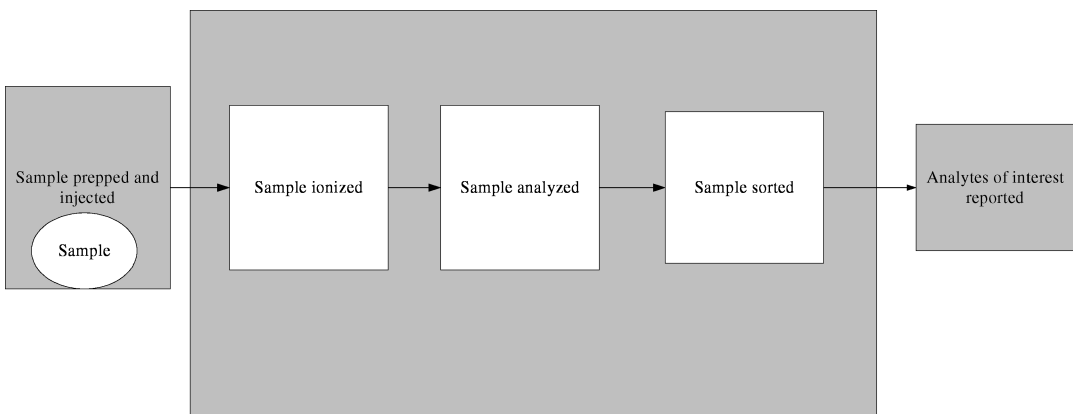


Figure 2. Simplified schematic of the components of a mass spectrometer.

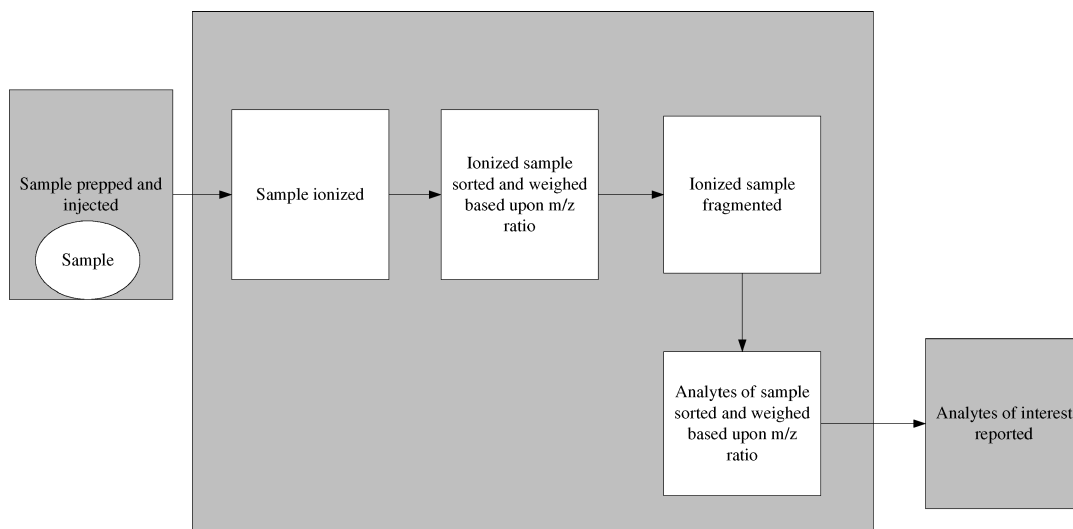


Figure 3. Simplified components of a tandem mass spectrometer.

analyte is compared to internal standard to yield both a qualitative and quantitative result. The result can be analyzed within minutes using sophisticated computer programs to produce histograms for analysis. The vertical lines of the histogram identify the mass while the horizontal axis represents the amount. The best analogy for this is a continuation of the example with pocket change. After entering the first instrument, the coins are sorted and weighed as dimes, pennies, nickels, and quarters. Next, the coins pass through the special chamber that further fragments them into the components of themselves. Pennies are broken down into copper and zinc while nickels, dimes, and quarters into copper and nickel. The analytes of copper, nickel, and zinc would be qualitatively and quantitatively reported, rather than the groups of dimes, nickels, pennies, and quarters. The MS/MS analysis works in much the same way.

TANDEM MASS SPECTROMETRY AND NEWBORN SCREENING

In a newborn's dried filter paper blood spot, the MS/MS typically tests for amino acids and acylcarnitines. Amino acids are the building blocks of protein for muscles, organs, tissue,

and blood. An amino acid is identified by an abbreviation of letters (Table 2). For example the amino acid, phenylalanine is phe. An elevated phe is associated with the disorder PKU. Carnitine is an ammonium compound, which has a key role in the transportation system for fats in and out of the mitochondria, the cell's energy factory.³⁵ When a fat is attached to a carnitine, it is an acylcarnitine.³⁶ An acylcarnitine is identified by the size of the fat molecule attached to it. These are categorized as short, medium, and long-chain acylcarnitine or are abbreviated as a combination of letters and numbers (Table 3). For example, the predominant short-chain fat attached to carnitine in the organic acid disorder of methylmalonic acidemia (MMA) is a 3 carbon acylcarnitine known as *propionyl* and is abbreviated C3.³⁷ The MS/MS can sort, weigh, and quantify this molecule and all the other desired acylcarnitines.

AMINO ACIDS AND ACYLCARNITINE PROFILES ON THE NEWBORN SCREEN

In errors of metabolism (EM), specific enzymes that breakdown amino acids or are needed in the conversion of fat to energy are either significantly decreased or absent. The

Table 2. Disorders associated with abnormal amino acid analyte levels from tandem mass spectrometry during the neonatal period*

Analyte	Name of compound	Change		Possible disorder to be considered
		↑	↓	
Arg	Arginine	X		Argininemia
Cit	Citrulline	X		Citrullinemia, ASA lyase deficiency
Leu/Ile	Leucine/Isoleucine	X		MSD
Meth	Methionine	X		Homocystinuria
Phe	Phenylalanine	X		Classical PKU and hyperphenylalaninemia variants
Tyr	Tyrosine	X		Tyrosinemia

Note: Citrullinemia indicates argininosuccinic acid (ASA) synthetase deficiency; ASA lyase deficiency, argininosuccinic acid lyase deficiency; MSD, Maple syrup disease; PKU, hyperphenylalaninemia.

*Adapted from Millington³⁵ and Northwest Regional Newborn Screening Program.³⁴

specific enzyme is not able to metabolize a compound. When a compound cannot be metabolized it begins to accumulate in the blood and tissues of the body. The compound becomes toxic and is a poison rather than a normal body substance. The MS/MS can measure the analytes of specific amino acids and acylcarnitines in the dried blood spot. When abnormal results are identified, the metabolic specialist discusses with the newborn's primary care provider how to proceed to confirm the presence of an EM.³⁸ Once samples for confirmatory studies are obtained, treatment may be started prior to results of confirmatory studies, which can take days or weeks in some cases, such as testing fibroblasts for carnitine transporter deficiency (CTD).³⁹⁻⁴¹ It is important to note that MS/MS cannot replace current programs for the screening for the EM disorders of biotinidase deficiency and galactosemia. These metabolic disorders cannot be identified by MS/MS at this time and must be detected by other methods such as fluorometric analysis.^{42,43}

DIAGNOSIS OF AMINO ACID DISORDERS BY MS/MS

Disorders of amino acid metabolism were some of the earliest EM investigated on a

large scale.¹ This was due to the availability of laboratory techniques to separate and identify amino acids in body fluids. In 1961, the development of a bacterial inhibition assay for phenylalanine measured from whole blood resulted in a reliable specific screening measure to identify newborns with PKU (Box 1).¹ The serum phenylalanine fluorometric procedure was modified to be a reliable screening test.⁵¹ The first report of MS/MS in the analysis of amino acid analysis was in 1991.⁵² This was followed by the use of MS/MS specifically to screen for PKU.²⁶ In the initial research report of amino acid analysis for PKU with MS/MS, newborn blood spot samples were obtained from normal infants, infants with confirmed PKU, and infants with false-positive results, that is those infants who screened positive, but in whom PKU was excluded to confirm the utility of MS/MS.²⁶ The concentrations of phenylalanine and tyrosine from MS/MS screening were compared to the concentrations obtained from fluorometric methods. The results were comparable. A subsequent study examined the interpretation of phenylalanine levels obtained in newborn filter paper spot samples collected at less than 24 hours of age comparing MS/MS and fluorometric analysis.⁵³ All newborns with confirmed classical PKU and variant

Table 3. Disorders associated with abnormal carnitine and acylcarnitine analyte levels from tandem mass spectrometry during the neonatal period*

Analyte	Name of acylcarnitine	Change		Possible disorder to be considered
		↑	↓	
C0	Carnitine (total)	X		CPTI
			X	CTD
C2	Acetyl		X	Carnitine Transporter Defect or Insufficiency
C3	Propionyl	X		MMA, PA, MCD
C3-DC	Malonyl	X		Malonic Aciduria
C4	Butyryl	X		SCAD deficiency, variant SCAD deficiency, Isobutyryl-CoA dehydrogenase deficiency, MAD
C4-DC	Methylmalonyl	X		MMA
C5	Isovaleryl	X		IVA, 2-methylbutyryl-CoA dehydrogenase deficiency, MAD
C5-DC	Glutaryl	X		GA-I, MAD
C5-OH	5-Hydroxyisovaleryl	X		3MCC, 3-methylglutaconyl CoA hydratase deficiency, HMG-CoA lyase, MCD, 2-methyl-3OH butyryl CoA dehydrogenase
C5:1	Tiglyl	X		3MCC, 2-methyl-3-OHbutyryl CoA dehydrogenase, SKAT, 3-Oxothiolase deficiency
C6	Hexanoyl	X		MCAD, MAD
C8	Octanoyl	XX		MCAD
C10	Decanoyl	X		MCAD
C10:1	Decenoyl	X		MCAD
C12	Dodecanoyl	X		MAD
C14	Tetradecanoyl	X		VLCAD
C14:1	Tetradecenoyl	X		VLCAD
C14:2	Tetradodocenoyl	X		VLCAD
C16	Palmitoyl	X		VLCAD, LCHAD, CPTII, CAT, MAD
			X	CPTI
C16-OH	3-Hydroxypalmitoyl	X		LCHAD, TFP
C18	Octadecanoyl	X		VLCAD
C18:1	Linoleoyl	X		VLCAD, CPTII, CAT, LCHAD, MAD
			X	CPTI
C18:1-OH	3-Hydroxylinoleoyl	X		LCHAD, TFP

Note: 3MCC denotes 3-methylcrotonyl-CoA carboxylase deficiency; CAT, carnitine/acylcarnitine translocase deficiency; CPTI, carnitine palmitoyl transferase I deficiency; CPTII, carnitine palmitoyl transferase II deficiency; CTD, carnitine transporter deficiency; HMG-CoA lyase, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; IVA, isovaleric acidemia; LCHAD, long chain L-3-hydroxyacyl CoA dehydrogenase deficiency; MAD, multiple acyl CoA dehydrogenase deficiency (also known as GA II, glutaric acidemia, type II and ethylmalonic-adipic aciduria); MCAD, medium chain acyl CoA dehydrogenase deficiency; MCD, multiple carboxylase deficiency; MMA, methylmalonic acidemia; PA, propionic acidemia; SCAD, short chain acyl CoA dehydrogenase deficiency; SKAT, mitochondrial acetoacetyl-CoA thiolase deficiency (also known as BKT, 3-ketothiolase deficiency, β ketothiolase); TFP, trifunctional protein deficiency; VLCAD, very long chain acyl CoA dehydrogenase deficiency.

*Adapted from Millington³³ and Northwest Regional Newborn Screening Program.³⁴

Box 1.**Hyperphenylalaninemia, Phenylalaninemia, Phenylketonuria—Alternative Names for PKU**

The most common disorder of amino acid metabolism is hyperphenylalaninemia, also known as *phenylalaninemia* or *phenylketonuria* (PKU). The incidence of PKU is approximately 1/10,000 to 1/25,000 live births.⁴⁴ The disorder occurs more frequently in people of Irish descent than in other ethnic groups.⁴⁵ PKU is an autosomal recessive disorder. The metabolic block in PKU is in the metabolism of phenylalanine. Phenylalanine cannot be converted to tyrosine in PKU (Fig 4).⁴⁶ The enzyme, phenylalanine hydroxylase is absent or significantly decreased. Excess phenylalanine accumulates in the blood and body fluids. The key to successful treatment of PKU has been early diagnosis with dietary restriction of phenylalanine in the diet.² The dietary restriction of phenylalanine results in a drop in the concentration of phenylalanine levels and the toxic metabolites disappear from body fluids. As a result, frequent quantitative assessment of the concentration of phenylalanine in the blood is needed for monitoring. The levels recommended as acceptable for phenylalanine have varied from 180 to 900 $\mu\text{mol/L}$. However, a tighter range between 120 and 300 $\mu\text{mol/L}$ showed a linear relationship between IQ and mean concentration over 300 $\mu\text{mol/L}$.⁴⁷ The difference was clear when phenylalanine levels exceeded 800 $\mu\text{mol/L}$ that IQ was negatively impacted. The assistance of a dietitian with experience in managing PKU is vital. Treatment requires the lifelong reduction of excessively elevated phenylalanine levels to as normal as possible.⁴⁵ There are now many low-protein foods available that are low in phenylalanine. Phenylalanine low-protein substitute foods facilitate improved long-term compliance with phenylalanine restriction within the therapy range. Despite this lifelong therapy, public policy has not addressed the coverage of medical foods and beverages. These foods are labeled for medical supervision and not typically available over-the-counter at the local grocery store due to the rarity of these conditions. Many states have passed legislation that mandates coverage of medical foods and beverages for the treatment of EMs, such as PKU.⁴⁸ The market for these foods are limited and can place a significant burden of the family as many of the major insurance providers will not cover medical foods and beverages, including phenylalanine-free formula needed by the newborn.⁴⁸ The reason for the legislative mandate was data clearly indicating that any deviation from a lifestyle of phenylalanine restriction will adversely impact neurophysiologic function and brain myelination. The result is lower IQ and increased behavioral problems.^{45,49,50} The savings to the state to support phenylalanine restriction is the long-term outcome of increased normal intelligence and behavior. Thus, the person with PKU is an active member of society, rather than dependent upon support due to lack of appropriate nutritional therapy. Preventive medicine in the form of medical foods and beverages lacking phenylalanine is the key.

forms of hyperphenylalaninemia, such as newborns with an increased phenylalanine level but not elevated enough to require medical nutritional therapy, were identified. In addition, the use of MS/MS decreased the number of false-positives from 91 to 3. Further analysis of the data using phenylalanine/tyrosine ratio (<2.5) reduced the number of false-positives to 1. Figure 5 illustrates the histogram from MS/MS profiles: one from a normal newborn screen and the other from

a newborn with confirmed PKU. Since these early studies with PKU, MS/MS has been used for analysis of other amino acids to identify other disorders of amino acid metabolism (Table 1).^{29,54,55}

DIAGNOSIS OF FATTY ACID OXIDATION DEFECTS BY MS/MS

Disorders of fatty acid oxidation compromise one of the most rapidly growing groups

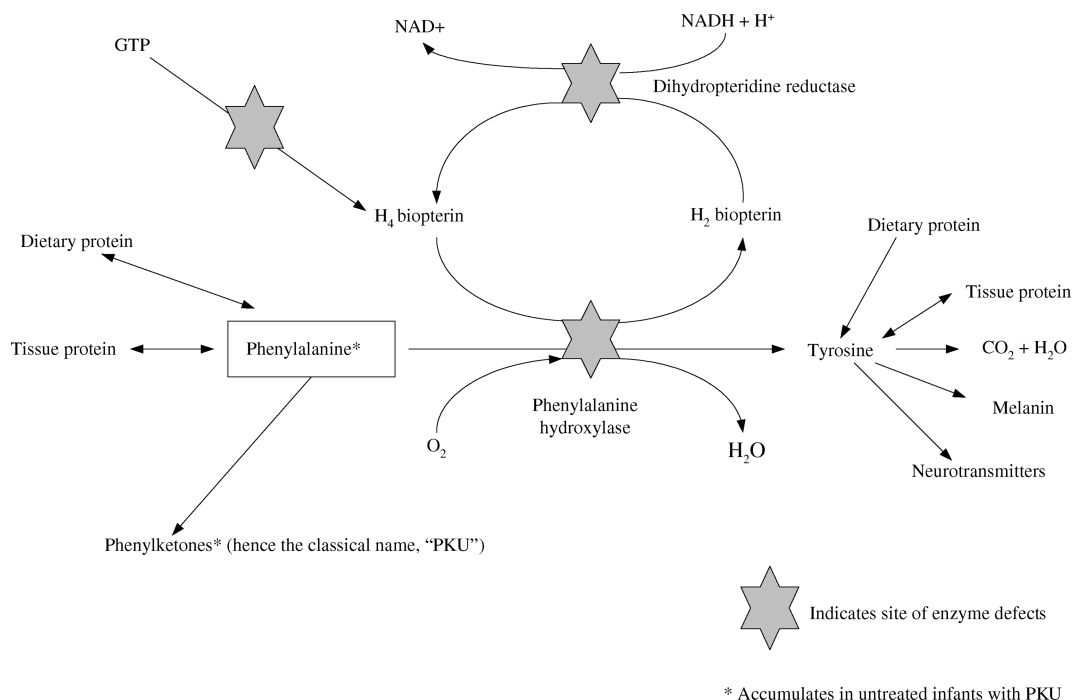


Figure 4. Simplified pathway of phenylalanine metabolism in hyperphenylalaninemia (PKU). PKU is the result of a defect in the enzyme phenylalanine (PHE) hydroxylase (PAH), which is responsible for converting phenylalanine (phe), an essential amino acid, to tyrosine (TYR), normally a nonessential amino acid. In classical PKU, there is a defect in PAH activity resulting in an accumulation of PHE in the blood and body tissues while TYR may be deficient. Thus, TYR becomes an essential amino acid for newborns with PKU. Tetrahydrobiopterin (H_4 biopterin) is a cofactor enzyme for PAH. H_4 biopterin deficiency is another rare cause of PKU.

of EM. As early as 1984, the diagnostic value of analysis of acylcarnitines was demonstrated.³⁶ Almost a decade later, the feasibility of using MS/MS for screening blood or plasma for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, the most common disorder of fatty acid oxidation, was explored (Box 2).²⁸ The initial diagnosis of MCAD deficiency was established by acylcarnitine analysis as an elevated C8 (octanoylcarnitine) concentration and a ratio of C8/C10 greater than 4 to 1. These same criteria were applied to a small set of neonatal blood spots retrieved from storage and successfully applied. A subsequent study verified the use of newborn dried blood spots from newborns with confirmed MCAD deficiency and healthy newborns.⁶⁷ In a prospective study, MS/MS re-

vealed elevated C8 levels in 9 infants.⁶⁸ The diagnosis of MCAD deficiency was confirmed in all 9 cases. Figure 7 illustrates the histogram from MS/MS profiles: one from a normal newborn screen and the other from a newborn with confirmed MCAD deficiency. MS/MS has been used for analysis of acylcarnitines for other fatty acid oxidation during the newborn period (Table 1).⁶⁹⁻⁷¹

DIAGNOSIS OF ORGANIC ACIDEMIAS BY MS/MS

The organic acid EM are disorders that less than 20 years ago were perplexing to understand. Physiologically many of these disorders share the same catabolic pathways

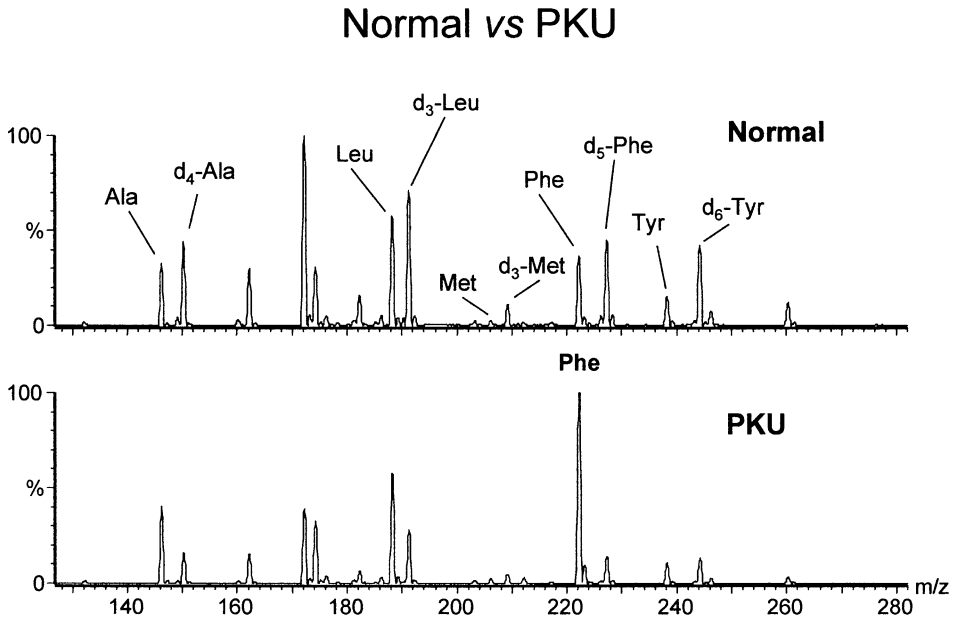


Figure 5. Amino Acid profile generated by MS/MS of normal blood (upper) compared with that of a newborn with hyperphenylalaninemia (lower). Permission from Waters Corporation (MS Technologies Centre).

Box 2.

Medium Chain Acyl-Coenzyme A Dehydrogenase (MCAD) Deficiency—More Common Than PKU

The most common disorder of fatty acid oxidation is medium chain acyl-CoA dehydrogenase (MCAD) deficiency.⁵⁶ The incidence of MCAD is approximately estimated at 1/6000 to 1/10,000 Caucasian births, making MCAD the most common EM disorder. MCAD deficiency is an autosomal recessive disorder and is characterized by episodic illness, such as vomiting and lethargy, associated with potentially fatal hypoglycemia. Metabolic decompensation occurs after fasting or catabolic stress, such as a fever. MCAD deficiency is associated with sudden unexpected death in infancy and after such minimal stresses as poor or childhood immunizations.⁵⁷⁻⁶² The enzyme, MCAD is one of the mitochondrial acyl CoA dehydrogenases that are needed to catalyze the initial steps in the beta-oxidation of fatty acids (Fig 6). Each of the enzymes within the beta-oxidation spiral is specific for only certain fatty acid chain lengths. MCAD accepts fatty acyl-CoAs within length of 6 to 12 carbons. The metabolic block in MCAD deficiency results in the inability to oxidize fatty acids to produce energy.⁶³ Treatment is simply the avoidance of fasting and the provision of caloric support during times of stress, such as childhood immunizations. Supplementation with carnitine is controversial. Initial studies with oral carnitine supplementation reported an increase in the conjugation and subsequent urinary excretion of acyl groups.^{64,65} As a result, there was an increase in the availability of free CoA and decreased acyl-CoA accumulation in the mitochondria. However, a subsequent study revealed findings that cast doubt on the value of long-term treatment with carnitine in patients with MCAD.⁶⁶ The supplementation of carnitine inhibited glycine conjugation, which is the major pathway for the disposal of C6 to C8 acylcarnitine analytes. No randomized, double-blind study has been done to answer the question if supplementation with carnitine is needed in patients with MCAD.

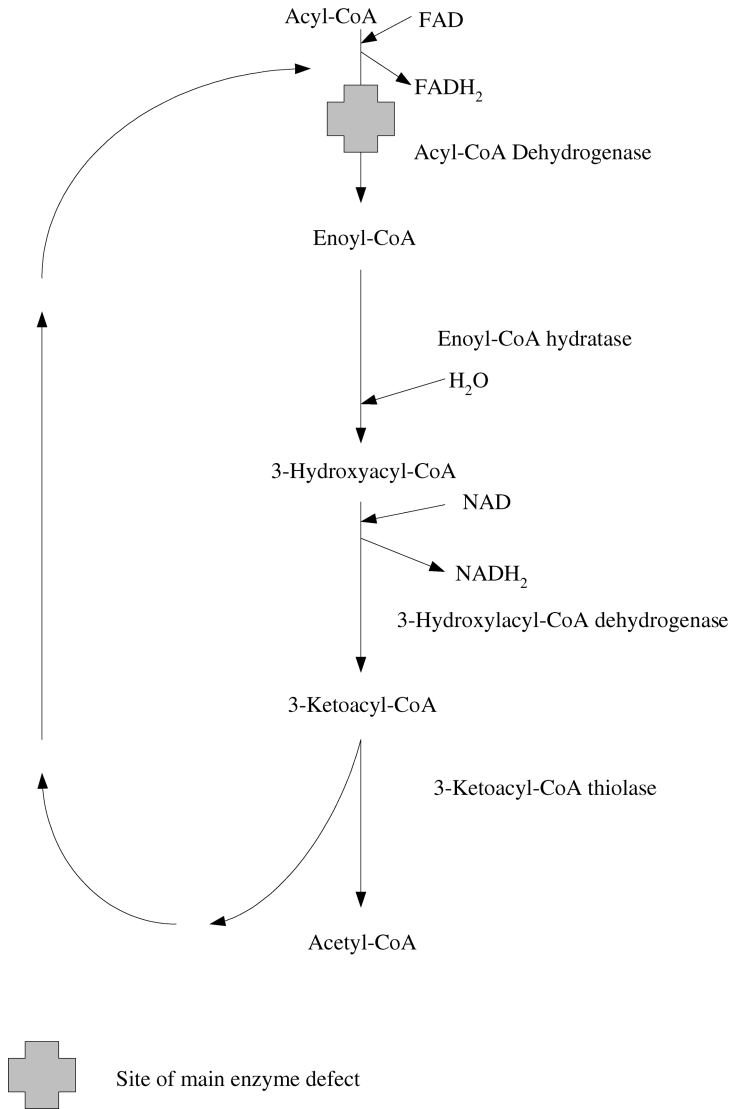


Figure 6. Simplified pathway of medium chain acyl-CoA dehydrogenase (MCAD) deficiency within the beta oxidation spiral of fatty acids.

as the branched-chain amino acids. The site of the enzymatic defect, however, is sufficiently removed from the step where the amino group is lost and does not accumulate. As a result, in the laboratory, the usual amino acid chromatographic analysis does not work for the identification of disorders of organic acid metabolism. With the development of gas chromatography-mass spectrometry, the unique metabolites of the errors of organic acid metabolism were identified.⁷²

It was an unusual odor of “sweaty feet” or “locker room odor” that was recognized by 2 chemists as that of a volatile short-chain organic acid. Subsequently, isovaleric acid was the first organic acidemia to be validated by gas chromatography.⁷³ Acylcarnitine profile of isovaleryl was determined from urine.³² This led to the acylcarnitine profiling of isovaleric acidemia (IVA) from newborn blood spots (Box 3).²¹ On MS/MS, IVA is considered when there is an elevated C5, isovaleryl.³³ An

Normal vs MCAD deficiency

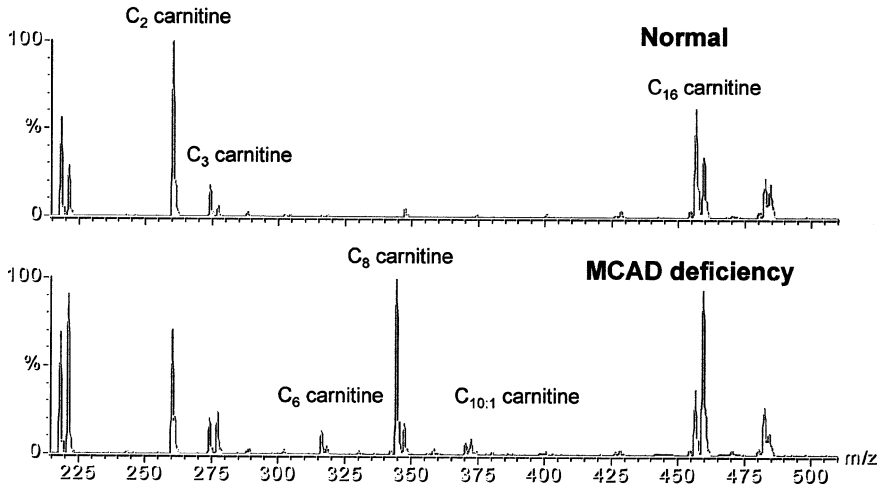


Figure 7. Acylcarnitine profile generated by MS/MS of normal blood (upper) compared with that of a newborn with medium chain acyl CoA dehydrogenase (MCAD) deficiency (lower). Permission from Waters Corporation (MS Technologies Centre).

elevated C5 that is twice the normal cutoff value should receive prompt follow-up to the primary care physician of that infant. Figure 9 illustrates the histogram from MS/MS profiles: one from a normal newborn screen and the other from a newborn with confirmed IVA. MS/MS has been used for analysis of acylcarnitines for other organic acidemias during the newborn period (Table 1).^{69,70}

ISSUES WITH MS/MS

More and more newborn screening programs across the United States and the world are adding MS/MS methodology to newborn screening. The “one test-one disorder” approach limited the number of disorders that could be screened. Typically, only 3 to 7 disorders would be screened dependent upon the state.⁹¹ However, with the development of MS/MS for the analysis of dried newborn blood spots for EM, testing has become more of a “one test-many disorders.” MS/MS has the capability of detecting between 20 and 40 disorders dependent upon the analytes being measured. With increased experience, the

number of disorders have increased—with the hope that morbidity, mortality, and mental retardation will be decreased. However, the natural history of many of these disorders are unknown as they have not been identified until recently in the neonatal period. In addition, not all of the disorders have been identified in newborns as some EM disorders may not have diagnostic analytes present in the early neonatal period.⁹² Many of the EMs have been identified in older children—thus facilitating care to be based upon diagnosis rather than symptoms and savings in treatment costs. This is hoped to be one of the major benefits of MS/MS—early diagnosis of EM disorders that will decrease the medical costs during the first 5 years of life.

With the automation of MS/MS, high-volume MS/MS screening for amino acids, fatty acid oxidation, and organic acid disorders became a reality by the mid-1990s. The initial reports of use of MS/MS in universal newborn screening demonstrated its use in large-scale newborn screening programs, such as the North Carolina Newborn Screening Program, the New England Newborn

Box 3.**Isovaleric Acidemia—One of the Most Common Organic Acidemias in the Neonatal Period**

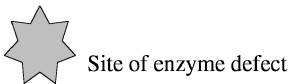
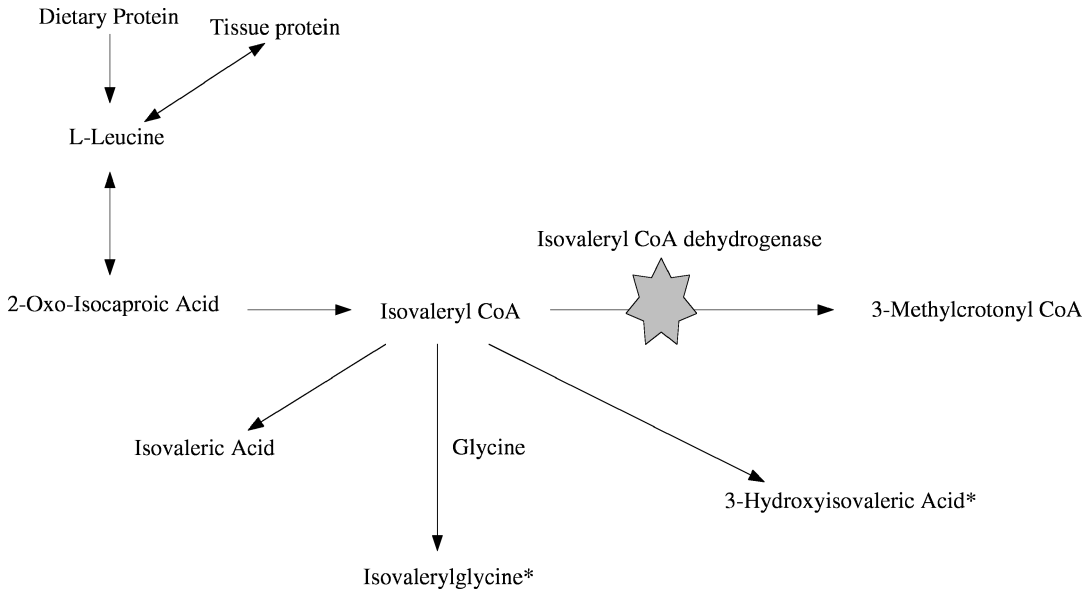
In the sick newborn, metabolic acidosis is a common finding. In some cases, the metabolic acidosis is acute, severe, and life threatening. While in other cases, the acidosis is mild but persistent, and in still others, it may be intermittent. Common treatment of metabolic acidosis in neonates is the administration of bicarbonate. Almost without exception, EM presenting acutely in the neonatal period with metabolic acidosis will not respond to bicarbonate, except transiently. The effective treatment of acidosis in EM is correction of the cause of the acidosis, such as decreasing the endogenous overproduction of specific acids.

The largest category of EM associated with metabolic acidosis during the neonatal period is organic acidemias.⁷⁴⁻⁷⁶ Isovaleric acidemia (IVA) is 1 of the 3 common organic acidemias to be considered in the evaluation of metabolic acidosis with an increased ion gap (>16) in the newborn.^{38,77} IVA was first reported in 1966.^{78,79} IVA is an autosomal recessive condition. The metabolic block in IVA is the result of defective branched chain amino acid metabolism, affecting the catabolism of isoleucine (Ile). The molecular block specifically in IVA is in the enzyme isovaleryl CoA dehydrogenase (Fig 8).⁸⁰ The typical presentation in IVA is a previously healthy newborn at birth who rapidly becomes ill after the first few days of life. Clinical signs and symptoms include ketoacidosis, poor feeding, vomiting, dehydration, hypotonia, lethargy, tachypnea/hypenpea, seizures, coma, and an usual odor, frequently described as “sweaty feet” or “locker room odor.”

The management of a newborn with a presumed diagnosis of an organic acidemia, especially IVA, is to first stabilize the newborn. Within 24 to 48 hours, the results of quantitative blood amino acid (QBAA), urine organic acid (UOA), and plasma acylcarnitine profile analysis should be available. If the laboratory cannot provide results in this time frame, alternative arrangements with another laboratory should be pursued. The UOA will be diagnostic for IVA with the presence of isovalerylglycine and 3-hydroxyisovaleric acid while the plasma acylcarnitine profile will demonstrate an elevation of C5, isovaleryl.⁸¹⁻⁸³ Treatment at this time is directed toward the removal of the accumulating metabolites, such as ammonia or organic acid intermediates, with hemodialysis.⁸⁴ If a center is unable to provide hemodialysis to a newborn, the newborn should be transferred. Exchange transfusions, peritoneal dialysis, or hemofiltration are less efficient than hemodialysis in managing these disorders.⁸⁵ Insulin can be used to augment the anabolic state.⁸⁶ Carnitine is used to remove toxic metabolites during the acute phase.⁸⁷⁻⁸⁹ During an acute illness, intravenous carnitine is preferred over oral administration. After the newborn has been stabilized, oral feedings should be reinitiated. A select range of specialty formulas are available that restrict certain amino acids during the neonatal period.⁹⁰ As with PKU, IVA is a lifelong diet of protein restriction. The assistance of a dietitian with experience in managing EM is vital. Frequent growth monitoring is needed to adjust the nutritional therapy plan. The success in the acute management IVA has led to improved survival and outcome. The long-term prognosis of IVA varies due to genetic heterogeneity, but is generally excellent when diagnosed and treated early.

Screening Program, and in Australia, where the technology was embraced.^{23,70,93} These programs, although successful, also emphasize some of the challenges with the use of MS/MS.^{94,95} These challenges include the establishment of cutoffs for the analytes to minimize the false-negatives without an excessive

amount of false-positives; the difficulty with definitive diagnosis for the newborn with an abnormal screen; and the increased number of newborns identified with an EM which must be followed up. Another challenge is that of the different newborn populations: healthy, premature or low birth weight, and



Site of enzyme defect

* Increased in UOA in IVA

Figure 8. Simplified pathway of isovaleryl CoA dehydrogenase, the site of the molecular defect in isovaleric acidemia (IVA). UOA indicates urine organic acids.

very low birth weight. Also, there is the need for consideration of total parenteral nutrition with and without carnitine supplementation; and collection times: at less than 24 hours of birth, 24 to 48 hours after birth, more than 48 hours but less than 7 days, more than 7 days but less than 14 days, and more than 14 days of age. Another challenge is that blood concentration of certain analytes change over time. One of the frustrations with MS/MS is the lack of specificity of certain analytes. An example is increased C3 (propionylcarnitine). The differential diagnosis of an elevated C3 includes propionyl-CoA carboxylase deficiency, also known as *propionic acidemia* (PA), methylmalonyl-CoA mutase deficiency, also known as *methylmalonic acidemia* (MMA), several cobalamin disorders, and dietary vitamin B₁₂ deficiency. Thus, specific biochemical tests are needed that many times are available only at large, academic teaching

institutions. The coordination of the newborn screening program with fast laboratory turnaround time, rapid intervention by the metabolic specialist, the ability of the primary care provider to locate the family to assess the newborn and obtain requested samples, and the confirmatory samples getting to the laboratory in a timely fashion for analysis is vital.

SUMMARY

With this exciting technology, MS/MS has increased the scope of newborn screening beyond the “one test-one disorder” system. With MS/MS, not only can disorders of amino acid metabolism be identified, but disorders of fatty acid oxidation and organic acidemias can also be rapidly identified with a dried newborn blood spot on filter paper. Theoretically, other EM disorders could be detected in the newborn period using MS/MS. These have yet

Normal vs Isovaleric acidaemia

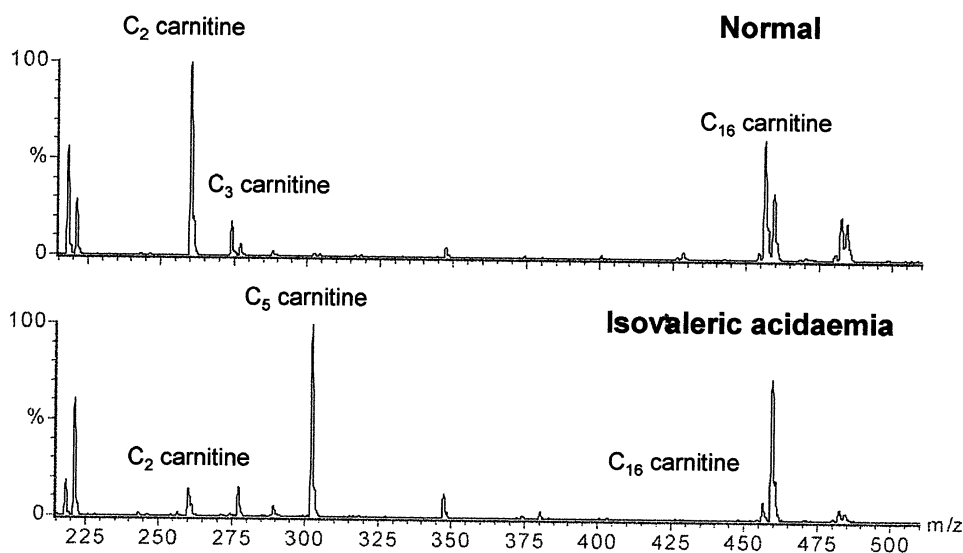


Figure 9. Acylcarnitine profile generated by MS/MS of normal blood (upper) compared with that of a newborn with Isovaleric acidemia (IVA) (lower). Permission from Waters Corporation (MS Technologies Centre).

to be identified. It is possible that some EM disorders may not have diagnostic analytes present in the early newborn period. Studies have validated that MS/MS decreases the number of false-positive results that can occur with older methods of testing. MS/MS allows the opportunity to identify many newborns with an EM prior to catastrophic insult, thus decreasing morbidity and mortality. In addition, MS/MS will allow improved

understanding of the natural history of many disorders, such as glutaric acidemia, type I with earlier identification of the newborn with specific EM. When newborn screening is done in a large, screening program with appropriate technical analytic chemistry and clinical biochemical genetic expertise, MS/MS provides a positive impact on the health of infants with an EM and their families.

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Tandem Mass Spectrometry in Newborn Screening: A Primer for Neonatal and Perinatal Nurses

Instructions:

- Read the article on page 41.
- Take the test, recording your answers in the test answers section (Section B) of the CE enrollment form. Each question has only one correct answer.
- Complete registration information (Section A) and course evaluation (Section C).
- Mail completed test with registration fee to: Lippincott Williams & Wilkins, CE Depart., 16th Floor, 345 Hudson Street, New York, NY 10014.
- Within 3–4 weeks after your CE enrollment form is received, you will be notified of your test results.
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CE TEST QUESTIONS

General Purpose: To provide registered professional nurses with the latest in-depth information on the use of tandem mass spectrometry in newborn screening.

Learning Objectives: After reading this article and taking this test, you will be able to:

- Outline the historical and scientific background pertaining to tandem mass spectrometry (MS/MS) in newborn screening.
- Discuss the use of MS/MS in newborn screening for particular disorders.
- Which of the following best describes the screening test for phenylketonuria (PKU) developed by Dr Robert Guthrie in 1961?
 - It was sensitive and used a bacterial inhibition assay.
 - It was expensive and used a bacterial inhibition assay.
 - It was sensitive and used paper chromatography.
 - It was simple, expensive and used a bacterial expression assay.
- All of the following are criteria proposed by Wilson and Jungner for adding a disease to the newborn screening test *except*
 - the frequency of occurrence is not a factor.
 - it should cause a documented spectrum of symptoms.
 - the disease should be treatable to a degree to decrease significant negative outcomes.
 - the appropriate confirmatory diagnostic tests must be readily available.
- Which statement about thin-layer chromatography for screening newborns is correct?
 - It could replace blood for all tests.
 - It had insufficient specificity and sensitivity.
 - Using urine facilitated specimen collection by parents.
 - Using urine eliminated false positive results.
- On what basic principle of physics is the mass spectrometer instrument based?
 - Analyzing numerous compounds simultaneously increases accuracy.
 - Analyzing numerous compounds simultaneously is more efficient.

- Every molecule has a unique mass.
 - Molecules are extremely small and cannot be weighed in the usual method on a scale.
- What are the roles of the two main instruments comprised by a tandem mass spectrometer?
 - The first sorts and weighs analytes; the second sorts and weighs molecules.
 - The first sorts and weighs analytes; the second fragments molecules into atoms.
 - The first sorts and weighs molecules; the second sorts and weighs analytes.
 - The first sorts and weighs molecules; the second breaks the sample into ions.
 - What are the building blocks of protein for muscles, organs, tissue, and blood?
 - Acylcarnitines.
 - Carnitines.
 - Free fatty acids.
 - Amino acids.
 - What causes errors of metabolism (EM)?
 - A compound that cannot be metabolized accumulates in blood and other tissues.
 - A compound is metabolized and then accumulates in blood and other tissues.
 - Specific fats are either significantly decreased or absent.
 - Specific enzymes are either significantly decreased or absent.
 - What is the input to a tandem mass spectrometer in newborn screening?
 - A dried blood spot
 - Urine
 - Amniotic fluid
 - Saliva
 - What is the next step after abnormal MS/MS results are identified?
 - Confirmatory studies are obtained.
 - Treatment is initiated.
 - Amino acids are identified.
 - Metabolites are quantified.
 - In the Chace et al (1998) study using MS/MS to screen newborns for PKU, what was the effect of analyses based on the phenylalanine/tyrosine ratio?
 - The number of false-negatives was reduced.
 - The number of false-positives was reduced.
 - The results were inconclusive.
 - The number of false-positives was increased.

- What is the most common disorder of fatty acid oxidation?
 - C8 deficiency
 - MDAC deficiency
 - C8/C10 deficiency
 - C8/C10 deficiency
- In the case of organic acid errors of metabolism, why doesn't the usual amino acid chromatographic analysis work?
 - The defect's site is unknown.
 - The defect's site is proximal to the step where the amino group is lost and does not accumulate.
 - The defect's site is removed from the step where the amino group is lost and does not accumulate.
 - The defect's site is proximal to the step where the amino group accumulates.
- How many disorders does MS/MS have the capability of detecting?
 - Between 12 and 40 disorders dependent upon the analytes being measured.
 - Between 2 and 14 disorders dependent upon the analytes being measured.
 - Between 20 and 40 disorders dependent upon the amount of blood being tested.
 - Between 20 and 40 disorders dependent upon the analytes being measured.
- Which of the following is *not* an issue emphasized by large-scale newborn screening programs, such as the North Carolina Newborn Screening Program and the New England Newborn Screening Program?
 - The establishment of cutoffs to minimize false-negatives without excessive false-positives.
 - The fact that blood concentrations of certain analytes change over time.
 - The lack of specificity of certain analytes.
 - Limitations of a "one test-one disorder" system.
- Which statement about PKU is correct?
 - Newborns with this disease have a characteristic odor described as "sweaty feet."
 - The enzyme phenylalanine hydroxylase is absent or decreased
 - It occurs more frequently in people of Asian descent than other ethnic groups.
 - It is an autosomal, dominant disorder.

CE Enrollment Form

Journal of Perinatal and Neonatal Nursing, January–March 2004: Tandem Mass Spectrometry in Newborn Screening: A Primer for Neonatal and Perinatal Nurses

A Registration information:

Last name _____ First name _____ MI _____
 Address _____
 City _____ State _____ Zip _____
 Telephone _____ Fax _____ email _____
 Registration Deadline: March 31, 2006
 Contact Hours: 2.5
 Fee: \$17.95

LPN RN CNS NP CRNA CNM Other _____
 Job Title _____ Specialty _____
 Type of facility _____
 Are you certified? Yes No
 Certified by _____
 State of License (1) _____ License# _____
 State of License (2) _____ License# _____
 Social Security _____
 From time to time we make our mailing list available to outside organizations to announce special offers. Please check here if you do not wish us to release your name and address.

B Test Answers: Darken one for your answer to each question.

- | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
|--------------------------|-----------------------|-----------------------|-----------------------|--------------------------|-----------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|-----------------------|
| 1. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 5. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 9. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 13. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| 2. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 6. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 10. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 14. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| 3. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 7. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 11. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 15. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| 4. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 8. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | 12. <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | | | | |

C Course Evaluation*

- | | A | B |
|--|------------------------------|-----------------------------|
| 1. Did this CE activity's learning objectives relate to its general purpose? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 2. Was the journal home study format an effective way to present the material? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 3. Was the content relevant to your nursing practice? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 4. How long did it take you to complete this CE activity? _____hours_____minutes | | |
| 5. Suggestion for future topics _____ | | |

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