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Using Tandem Mass Spectrometry for Metabolic Disease Screening Among Newborns

A Report of a Work Group

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Contents

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Using Tandem Mass Spectrometry for Metabolic Disease Screening Among Newborns

A Report of a Work Group

"Public health agencies (federal and state), in partnership with health professionals and consumers, should continue to develop and evaluate innovative testing technologies [and] design and apply minimum standards for newborn screening activities . . . ."

Newborn Screening Task Force, May 1999

Summary

Increasingly, tandem mass spectrometry (MS/MS) is being used for newborn screening because this laboratory testing technology substantially increases the number of metabolic disorders that can be detected from dried blood-spot specimens. In June 2000, the National Newborn Screening and Genetics Resource Center, in collaboration with CDC and the Health Resources and Services Administration, convened a workshop in San Antonio, Texas. Workshop participants examined programmatic concerns for health providers choosing to integrate MS/MS technology into their newborn screening activities. Representatives from approximately 50 public and private health agencies and universities participated in the workshop. The workshop participants and work group focused on laboratory methodology, decision criteria, quality assurance, diagnostic protocols, patient case management, and program evaluation for using MS/MS to analyze dried blood spots routinely collected from newborns. This work group report contains proposals for planning, operating, and evaluating MS/MS technology in newborn screening and maternal and child health programs. As a supplement to these proposals, this report contains synopses of selected presentations made at the 2000 workshop regarding integration of MS/MS technology into newborn screening programs. The proposals contained in this report should assist policymakers, program managers, and laboratorians in making informed decisions regarding the process of including MS/MS technology in their newborn screening and maternal and child health programs.

INTRODUCTION

Each year, approximately 4 million babies in the United States have dried blood spots analyzed through newborn* screening programs. This screening is intended to detect inborn disorders that can result in early mortality or lifelong disability. Detectable disorders include metabolic disorders (e.g., phenylketonuria [PKU]), hematologic disorders (e.g., sickle cell disease), and endocrinopathies (e.g., congenital hypothyroidism). These three groups of disorders account for approximately 3,000 new cases of potentially fatal

* *Newborn* is defined as an infant aged ≤1month.

or debilitating disease each year for which outcomes are improved with early identification and treatment through newborn screening systems. The introduction of tandem mass spectrometry (MS/MS) in the 1990s for population-based newborn screening has enabled health-care providers to detect an increased number of metabolic disorders in a single process by using dried blood-spot specimens routinely collected from newborns (*1–3*). However, using MS/MS in newborn screening programs is new, and scientific data are limited regarding incorporating this technology into newborn screening and maternal and child health programs.

MS/MS technology enables improvements in and consolidation of metabolic screening methods to detect amino acid disorders (e.g., PKU, maple syrup urine disease, and homocystinuria) among newborns, and does so with a low false-positive rate (*4–6*). MS/MS technology expands the metabolic disorder screening panel (i.e., the number of disorders that can be detected) by incorporating an acylcarnitine profile, which enables detection of fatty acid oxidation disorders (e.g., medium-chain acyl-CoA dehydrogenase [MCAD] deficiency) (*7–10*) and other organic acid disorders. MS/MS can reliably analyze approximately 20 metabolites in one short-duration run (i.e., ~2 minutes) and provide a comprehensive assessment from a single blood-spot specimen (Table 1).

Screening for multiple disorders in a single analytical run by using MS/MS requires that program administrators and laboratorians choose which types of conditions are to be screened. For example, laboratory A uses MS/MS to detect amino acids only; laboratory B uses MS/MS to detect acylcarnitines only; and laboratory C screens for both. In addition, other technical concerns must be addressed before MS/MS technology can be integrated effectively into a newborn screening program, including deciding which analytes to use in characterizing each disorder (e.g., octanoylcarnitine analysis can indicate both MCAD deficiency and multiple acyl-CoA dehydrogenase deficiency).

Studies are limited regarding use of MS/MS technology in newborn screening programs, but existing studies indicate that screening a full panel of acylcarnitines and amino acids yields rates of 1:4,000–1:5,000 for MS/MS-detectable disorders (*11–13*). For certain metabolic disorders, early detection can result in substantial improvements in health outcomes. For example, MCAD, which has an incidence rate of 1:10,000–1:20,000 newborns, results in substantial morbidity and reported mortality rates of 20%–25% among infants and children during the first 3 years of life (*14*). Effective treatment is available, and detection and intervention before onset of illness can prevent mortality and improve the quality of life for MCAD patients. Although effective treatments do not exist yet for certain other metabolic disorders identifiable by MS/MS testing (*1*), patient and family advantages can still be achieved through early diagnosis (*15*). Also, MS/MS can detect metabolic disorders after an illness occurs, even if that illness occurs after the newborn period (Table 2).

June 2000 Workshop and Work Group Goals

In June 2000, the National Newborn Screening and Genetics Resource Center, in collaboration with CDC and the Health Resources and Services Administration (HRSA), convened a workshop in San Antonio, Texas, to examine programmatic concerns for effectively integrating MS/MS technology into newborn screening programs. Representatives from approximately 50 public and private health agencies and universities participated in the workshop.* The participants focused on laboratory methodology, decision

* The proposals in this report are based on conclusions derived by participants in the plenary and work group sessions held during the workshop.

TABLE 1. Metabolic disorders detectable in newborns aged 1–5 days by using tandem mass spectrometry

Disorder	Primary metabolic indicator
Amino Acids	
Phenylketonuria	Phe
Maple syrup urine disease	Leu/Ile, Val
Homocystinuria (cystathione synthase deficiency)	Met
Hypermethioninemia	Met
Citrullinemia	Cit
Argininosuccinic aciduria	Cit
Tyrosinemia, type I	Tyr
Fatty Acids	
Medium-chain acyl-CoA dehydrogenase deficiency	C8, C10, C10:1, C6
Very-long–chain acyl-CoA dehydrogenase deficiency	C14:1, C14, C16
Short-chain acyl-CoA dehydrogenase deficiency	C4
Multiple acyl-CoA dehydrogenase deficiency	C4, C5, C8:1, C8, C12, C14, C16, C5DC
Carnitine palmitoyl transferase deficiency	C16, C18:1, C18
Carnitine/acylcarnitine translocase defect	C16, C18:1, C18
Long-chain hydroxy acyl-CoA dehydrogenase deficiency	C16OH, C18:1OH, C18OH
Trifunctional protein deficiency	C16OH, C18:1OH, C18OH
Organic Acids	
Glutaric acidemia, type I	C5DC
Propionic acidemia	C3
Methylmalonic acidemia	C3
Isovaleric acidemia	C5
3-hydroxy-3-methylglutaryl CoA lyase deficiency	C5OH
3-methylcrotonyl CoA carboxylase deficiency	C5OH

Notes: The list of primary metabolic indicators is not all-inclusive and serves only as a guideline. It is based on results obtained from laboratories experienced in tandem mass spectrometry technology and that serve as diagnostic metabolic laboratories in the United States and other countries. The identified disorders have been detected from analyses of dried blood-spot specimens collected during the newborn period. Certain disorders require complex metabolic profiles and intermetabolic relation to detect disease with low false-positive and no false-negative rates.

criteria, quality assurance, diagnostic protocols, patient case management, and program evaluation. This report contains their proposals for planning, operating, and evaluating MS/MS technology in newborn screening and maternal and child health programs. Their proposals are included in this report to assist policymakers, program managers, and laboratorians in planning state-mandated screening programs or optional metabolic testing through partnering of state and private screening laboratories. The workshop participants did not address newborn disorders that are screened by other technologies and that should be considered for a comprehensive newborn screening panel (e.g., sickle cell disease, congenital adrenal hyperplasia, galactosemia, biotinidase deficiency, and cystic fibrosis). Therefore, these proposals address MS/MS technology only. Further, as a supplement to their proposals, this report contains synopses of selected presentations made at the 2000 workshop regarding integration of MS/MS technology into newborn screening programs (Appendix).

Specifically, workshop participants focused on the following concerns for policymakers, program managers, and laboratorians interested in MS/MS testing for newborn screening programs:

- What barriers exist that impede MS/MS implementation?

TABLE 2. Metabolic disorders detectable in patients aged >5 days by using tandem mass spectrometry

Disorder	Primary metabolic indicator
Argininemia	Arg
Nonketotic hyperglycinemia	Gly
Tyrosinemia, type II	Tyr
Hyperammonemia, hyperornithinemia, homocitrullinuria syndrome	Orn, HomoCit
5-oxoprolinuria	5-Oxopro
Carnitine palmitoyl transferase, type I deficiency	Free CN
Isobutyrl CoA dehydrogenase deficiency	C4
Mitochondrial acetoacetyl CoA thiolase deficiency	C5:1,C5OH
Malonic aciduria	C3-DC
2-methylbutyrl CoA dehydrogenase deficiency	C5

Notes: The list of primary metabolic indicators is not all-inclusive and serves only as a guideline. The identified disorders have been detected by tandem mass spectrometry as a result of follow-up testing conducted because of illness or for older children with suspected underlying metabolic disorders. Disorders listed might not be detectable during the newborn period. Many disorders require complex metabolic profiles and intermetabolic relation to detect disease with low false-positive and no false-negative rates.

- What technical concerns exist regarding instrumentation (e.g., throughput, cost, software, accessories, or capabilities)?
- What are the approaches and cutoff decisions for identifying presumptive positive test results?
- What guidance do program managers and laboratorians need who are planning to use, are using already, or are evaluating MS/MS technology for newborn screening?
- What are the expectations and resource needs for follow-up, diagnostic confirmation, parental genetic counseling, and patient case management?
- What are the medical concerns for parents and health-care providers, including identification and treatment of MS/MS-diagnosed disorders?
- How would program evaluation be conducted for MS/MS integration, including quality control and proficiency testing?

In their discussions, workshop participants considered the role parent support groups* and advocacy organizations are taking in promoting inclusion of MS/MS technology in newborn screening services. This increased advocacy has resulted in increased media attention regarding the health burden of metabolic disorders among newborns (*16,17*). Consequently, more newborn screening program administrators and maternal and child health-care providers are considering integrating this technology into their programs (*18*). Key factors in deciding to implement MS/MS are its versatility and ability to detect additional treatable metabolic diseases, including fatty acid and organic acid oxidation disorders. However, medical literature is limited regarding use of MS/MS technology in newborn screening programs. Furthermore, the identification of metabolic disorders

* Example parent support groups are located at the following Internet sites: <http://www.pku-allieddisorders.org> (accessed March 12, 2001). <http://www.tylerforlife.com> (accessed March 12, 2001).

must be confirmed by validated scientific methodologies. Additional studies are needed to assess effectiveness of MS/MS, and sustained discussions among those persons involved in using or contemplating using MS/MS should be ongoing to enable policymakers, program managers, and laboratorians to make more informed decisions.

BACKGROUND

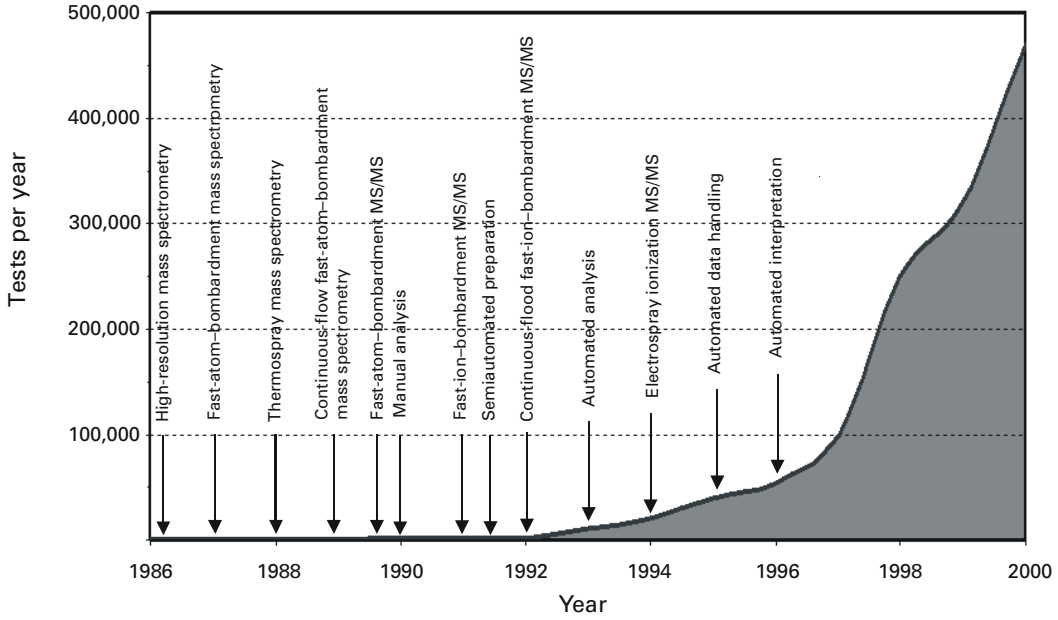
In the 1930s, amino acid metabolism disorders and in the 1970s, fatty acid oxidation disorders were recognized as causes of morbidity and mortality among infants and children. As metabolic disorders were recognized, researchers worked to develop methods to detect them. In the 1960s, population screening for amino acid disorders began, and in the mid-1980s, one researcher demonstrated the therapeutic value of measuring the fatty acids released from acylcarnitines (*19*). Early analytical methods required hydrolysis of fatty acids from acylcarnitines or analysis of urinary organic acid profiles as primary analytical approaches to diagnosing inborn errors of metabolism (*20,21*). Acylcarnitines are quaternary ammonium salts that are not readily analyzed by gas chromatography/mass spectrometry, a readily available clinical chemistry method. With the introduction of fast-atom–bombardment ionization techniques for MS, ionic compounds with high polarity (e.g., acylcarnitines) could be analyzed with simple preparation techniques (*22*). Use of MS/MS for acylcarnitine analytes in plasma eliminated time-consuming chromatography but maintained method specificity (*23,24*). Rapid analyses of highly polar compounds made possible use of MS/MS in newborn screening, which requires rapid high throughput to be cost-effective. Also, during the 1980s, improved diagnostic skills enabled better recognition of disorders of intermediary metabolism. In 1990, analyses of amino acids present in dried blood-spot specimens were used to document newborn screening applications of MS/MS (*4,5,10,25*). Existing methodology for the MS/MS analyses of acylcarnitines was modified and combined with that for amino acids in an approach that remains relatively unchanged.

During the early 1990s, improvements were made in automated analysis, in part enabled by the introduction of electrospray ionization, sample-introduction techniques, method validation, and development of automated interpretation systems (*8,9,11,12,26–29*). Concurrently, the number of specimens analyzed annually increased substantially. From the first analyses in 1990 of 5–10 specimens/day in clinical testing laboratories to early pilot studies in 1993 of 60–120 specimens/day (*11*), the use of MS/MS technology has grown exponentially so that, during 2000, an estimated 500,000 specimens* were analyzed (Figure 1).

With increased demand for expanded newborn screening, MS/MS technology has been successfully implemented in private sector and public health laboratories across the United States (Figure 2). Routine, state-sponsored screening using MS/MS is performed in the District of Columbia, New England (i.e., Massachusetts, New Hampshire, Vermont, Maine, Rhode Island), North Carolina, and Wisconsin. However, the technology is used for screening acylcarnitine profiles in only three of these states: Massachusetts, North Carolina, and Wisconsin. In addition, optional supplemental testing (i.e., testing for diseases not included in the selected panel of a state screening program) is offered for a

*This estimate is a summation of the known birth rate for the states where MS/MS technology was used for newborn screening in 2000.

FIGURE 1. Progress leading to the introduction of tandem mass spectrometry (MS/MS) in newborn screening



fee by Neo Gen Screening, Inc.* (Bridgeville, Pennsylvania) and the Institute of Metabolic Disease† (Baylor University Medical Center, Dallas, Texas) to parents, physicians, and hospitals either directly or through state newborn screening programs. Pilot testing is under way in Illinois, Iowa, Louisiana, and Ohio; and California, Minnesota, and New York have purchased MS/MS equipment.

LABORATORY PRACTICE

Policymakers and program managers need to be familiar with standards for MS/MS testing when soliciting, contracting, or evaluating newborn screening laboratory services. Work group proposals regarding implementation and operation of MS/MS laboratories are provided here with the understanding that this technology is new and, therefore, changing rapidly. Although modifications throughout the testing system are to be expected, all changes in laboratory practice should adhere to published laboratory guidelines before being implemented.

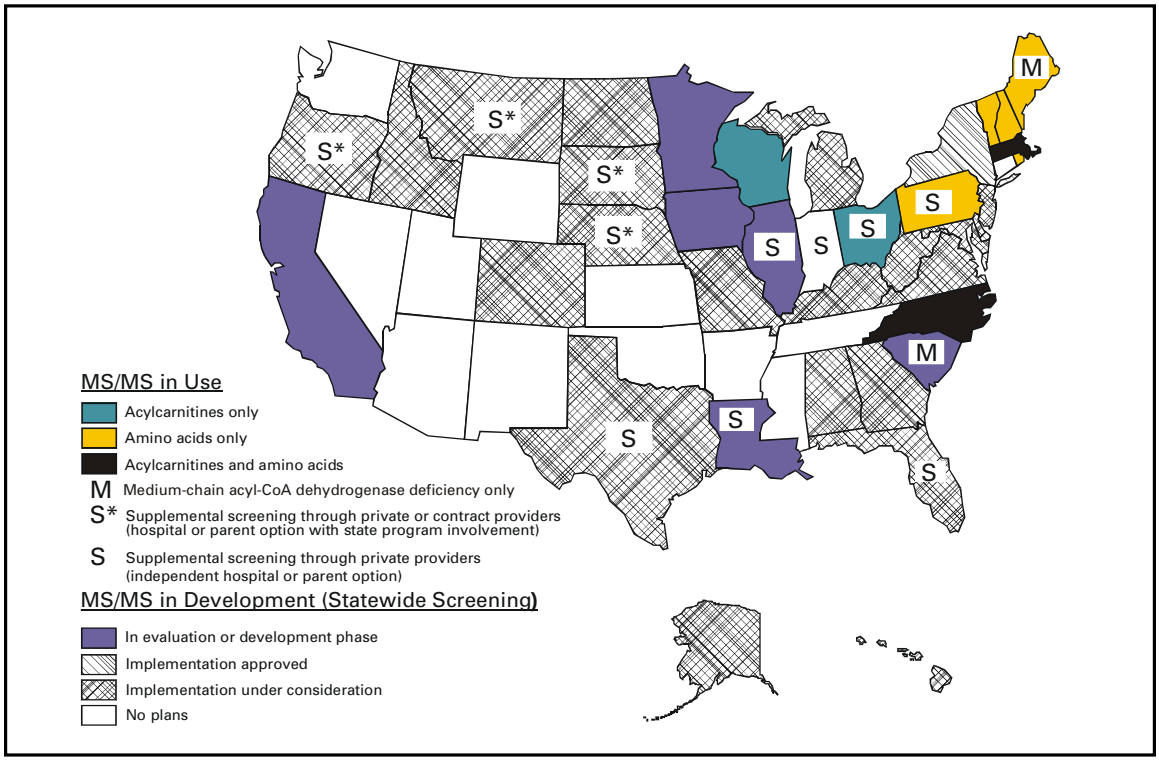
Nomenclature

Basic knowledge of MS/MS technology standards and laboratory practice requires an understanding of the scientific terminology.

*Additional information is available at <http://www.neogenscreening.com> (accessed January 17, 2001).

†Additional information is available at <http://www.baylordallas.edu/newbornscreening> (accessed February 16, 2001).

FIGURE 2. Tandem mass spectrometry (MS/MS) integration into newborn screening programs — United States, December 2000



Work Group Proposals

- The technology should be referred to as *tandem mass spectrometry*, not *tandem mass spectroscopy*.
- The standard abbreviation for tandem mass spectrometry is *MS/MS*, not *TMS* or *TM/TM*. *MS/MS* represents two mass spectrometers joined by a fragmentation chamber.
- A universal description of the analytes and disease states is needed. For example, *succinate* and *methylmalonate* can refer to the same analyte. *MCAD* and *MCADD* are used interchangeably to refer to medium-chain acyl-CoA dehydrogenase deficiency. A task force of laboratorians and clinical specialists should work to resolve such nomenclature differences.
- Acylcarnitine results should be reported in micromolar (µM) units (whole blood), and amino acids should be reported as micromolar (µM) (whole blood) with the concentration in milligrams per deciliter (mg/dL) given in parentheses.

Standard and Sample Preparation

Work Group Proposals

- Sample preparation techniques for acylcarnitines and amino acids should be validated in accordance with the Clinical Laboratory Improvement Amendments of 1988 (*30*) and good laboratory* and measurement† practices (*31*).
- All reagents, buffers, and solvents should be high-pressure liquid chromatography grade or better.
- Validated methods should be published in peer-reviewed journals.
- When stable-isotope internal-standard methodology is used, the labeled internal standard should be identical to the analyte of interest. If the analyte is not available in the labeled form, the nearest homologue can be substituted. For example, the diagnostic analyte is phenylalanine for PKU, which requires D_5-phenylalanine, or equivalent, for quantitation. However, not all acylcarnitines will be available as internal standards for detecting fatty acid oxidation or organic acid oxidation disorders by measuring acylcarnitines levels. Therefore, for a complete acylcarnitine profile, D_9-C0, D_3-C3, D_3-C4, D_9-C5, D_3-C14, and D_3-C16 should be used as internal standards. Other isotopes can be used if they are properly validated. Limited profiling might involve fewer internal standards but still requires validation.
- Individual and premixed stable-isotope internal standards are commercially available. Commercial suppliers should include a statement regarding the standard material's concentration and stability. Dilutions of commercial standards or in-house preparations should be validated by analyzing unlabeled materials (i.e., controls) before use.
- Stability of internal-standard or quality-control preparations should be validated and documented for each laboratory performing MS/MS analyses.
- Testing protocols should specify what safety recommendations, universal precautions, personal protective gear, and environmental controls are required.
- Sample preparation areas should be physically separated from instrument areas to avoid contamination. One MS/MS instrument should have a capacity of 500 samples/day. Good laboratory and measurement practices should be followed (*31*).

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* *Good laboratory practice* is defined as an acceptable way to perform a basic activity that is known to influence the quality of its output.

† *Good measurement practice* is defined as an acceptable way to perform an operation with a specific measurement technique known to influence the quality of the measurement.

Instrument Resources and Calibration

Work Group Proposals

- Manufacturer's guidelines for power requirements, exhaust specifications, laboratory gas purity and pressure, and laboratory environment should be followed. All MS/MS used for metabolic disease detection requires high-purity compressed nitrogen delivered at a specified pressure. This gas can be supplied by nitrogen generators, compressed gas tanks, or Dewar tanks. Certain instruments require uninterrupted gas flow; therefore, the ability to change tanks regularly without interruption of flow or loss of pressure is required (i.e., empty tanks connected to the system must be replaced with filled tanks before all tanks become empty).
- Additional peripheral equipment for MS/MS is required, including a liquid chromatography pump, syringe pump for calibrating and tuning, and an autosampler (preferably one capable of holding multiple 96-well microplates). Sample preparation equipment might include sample concentrators, forced air ovens or equivalent, and reagent-dispensing devices (either automated or hand-held). Nearby telephone access is desirable for instrument troubleshooting.
- Operators of MS/MS instruments should hold a minimum of a bachelor of science degree in a laboratory science or medical technology. In addition, they must meet the pertinent Clinical Laboratory Improvement Amendments of 1988 personnel requirements (*30*). Additionally, MS/MS laboratorians should have a) mechanical aptitude, b) computer skills, and c) an interest in mass spectrometry technology. Each instrument requires one primary laboratorian and a backup. Laboratories having multiple instruments should have an equal number of personnel plus one or two laboratorians, depending on whether the supervisor serves as the backup.
- Managers and supervisors of MS/MS operations should have background experience in mass spectrometry. One manager is sufficient to oversee multiple instruments.
- Newborn screening laboratories should develop a backup plan for instrument downtime. That plan should include ready access to additional instruments or backup laboratories.
- Each instrument manufacturer's recommended calibration procedure should be followed. These procedures involve using a standard material (e.g., sodium iodide, rubidium iodide, polypropylene glycol, or similar compound).
- Mass scales should be calibrated above and below the mass range of interest. Calibration from 23 megahertz to 600 megahertz should suffice for analyses being performed in metabolic disease investigations.
- Users should prepare an instrument check solution at a defined concentration comparable to patient specimens in mass and intensity. This solution should be used daily to measure the sensitivity of the instrument. Results, as counts or signal intensities, should be recorded each day and a minimum signal intensity established as an alert of a possible change in instrument performance. Check

solutions should contain the same mix of standards used to calibrate the assay. A higher concentration tuning solution should be available also and separate from the routine check solution. This tuning solution enables optimization of parameters for individual acylcarnitines that might have variable ionization efficiencies of short- versus long-chain acylcarnitines and neutral versus basic amino acids.

Reducing Instrument-to-Instrument Variability

Work Group Proposals

- Because quantitative results for raw ion counts can vary from instrument to instrument, a minimum sensitivity threshold for all instruments should be defined before use. Concentration calculations using ion ratios (i.e., the mass of unlabeled analyte versus labeled internal standards) should vary ≤10% from instrument to instrument. NCCLS* voluntary consensus definitions of quantitation and detection limits should be used (*32*).
- When the concentration of an analyte is close to the detection limits of the method, as for certain acylcarnitines, differences between apparent normal results from one instrument to another can result from electronic signal noise. This difference should decrease as a particular analyte level increases and should be minimal in true disease states when multiple instruments are properly maintained.

Quality Control, Proficiency Testing, and Quality Assurance

Work Group Proposals

- Quality control should consist of ≥2 control specimens/96-well microplate. One control should contain analyte concentrations above the abnormal reporting level, and the second control should be at or near the abnormal cutoff value. The fatty acid oxidation disorder and organic aciduria disorder controls should contain as many of the acylcarnitines as are commercially available.
- A reagent blank should be included on each plate and should be located after the high-level control to monitor analytical carry-over.
- The daily patient mean, or median, for each analyte should be monitored to follow method performance.
- A schedule for routine maintenance should be established for quality performance. Maintenance schedules should follow manufacturers' recommendations and each instrument's operational experience.

* Formerly the National Committee for Clinical Laboratory Standards.

- External quality-control specimens should be analyzed periodically. CDC's Newborn Screening Quality Assurance Program, operated in collaboration with the Association of Public Health Laboratories, can provide control materials for amino acids and acylcarnitines.* For acylcarnitines, the calculated concentrations are dependent on the internal standard to which the unlabeled acylcarnitine is compared. Therefore, when interlaboratory comparisons are performed, the internal standards used to calculate the reported values need to be defined. Using a relative response factor by dividing each laboratory's observed value by the expected value might be advantageous when interlaboratory performances are compared.
- A government-supported external proficiency testing program is needed for newborn screening laboratories using MS/MS testing (*1*). CDC's Newborn Screening Quality Assurance Program is pursuing addition of a dried blood-spot proficiency testing program for MS/MS-detectable disorders. Proficiency testing challenges should include quantitation of analytes and assessment of the laboratory's capability to recognize disease profiles. CDC's program is producing dried blood-spot materials for amino acids and acylcarnitines quality control and proficiency testing purposes, but problems remain with standardization of such analytes as glutarylcarnitine and hydroxyacylcarnitines for which no synthetic material is available. When external proficiency testing is not available, an interlaboratory specimen exchange program with documented results should be established to assess accuracy in detecting metabolic disease profiles.
- Results of a newborn screening test might be affected by the patient's medical treatment before specimen collection. Research is needed regarding the effect of carnitine-fortified total parenteral nutrition and hyperalimentation solutions, transfusions, prescription drugs, and metabolites that can affect the MS/MS test results. Discrepancies among multiple sample collections should be discussed with metabolic specialists, physicians, and the patient's primary care provider.
- MS/MS, although specific and accurate, is one of multiple tests performed in newborn screening laboratories. As with other tests, abnormal results should be confirmed by additional diagnostic testing, including MS/MS serum analysis, gas chromatography/mass spectrometry urinary organic acid analysis, or quantitative high-pressure liquid chromatography using ion exchange or similar approaches, depending on disease confirmation.
- An electronic communication system (e.g., an on-line bulletin board service) should be used for information exchange among laboratories regarding MS/MS operational issues, problems, and solutions.†

* Additional information is available at <http://www.cdc.gov/nceh/dls/newborn_screening.htm> (accessed March 12, 2001).

† Readers can subscribe to the following electronic bulletin board, <MSMSWG@lists.uthscsa.edu>, by contacting Donna Williams at <williamsdc@uthscsa.edu> and requesting a membership application.

- The Association of Public Health Laboratories, in collaboration with other organizations, should sponsor a regularly held workshop (e.g., annually) for newborn screening MS/MS instrument users. In addition, a session regarding MS/MS should be included at national and regional conferences on newborn screening.

Interpreting MS/MS Data and Reporting Results

Work Group Proposals

- Cutoff values for reporting abnormal levels for each analyte should be established by using statistical measurements (e.g., percentiles, means, and standard deviations) in consultation with metabolic disease specialists. A multitier system could be designed, depending on methodology, metabolic disorder, and target population. Cutoff values should be compared with published ranges but should be individualized to the methodology used and patient population. Cutoff values should be adjusted up or down, on the basis of periodic re-evaluations or changes in methodology or population distribution. Unless interinstrument performance comparability is $\leq$10% (i.e., concentration calculations), instrument-specific cutoff values should be developed. Programs with multiple instruments should be able to perform satisfactorily for a single cutoff value.
- Laboratories can elect to establish two levels of abnormal results. One level would be the concentration that is indicative of a particular disorder. Test results greater than that concentration would require immediate referral to the clinical management team for follow-up. The second level would be a borderline concentration that would require resampling and retesting. Decisions to use this two-level system might be dependent on the availability of follow-up resources.
- In addition to defining analyte concentrations or to profiling analytes, a ratio of different analytes might be helpful in data interpretation. For example, the phenylalanine/tyrosine ratios and C8/C10 acylcarnitine ratios might reduce false-negatives and false-positives in PKU and MCAD detection, respectively.
- In addition to establishing a high cutoff value, setting a low cutoff value for certain analytes would be useful. For example, a low, free carnitine might mask the presence of a disorder (i.e., if low or undetectable carnitines are present, acylcarnitines cannot be formed).
- For specimens collected from newborns aged >1 week, the acylcarnitine measurements should be examined closely because acylcarnitines levels decrease significantly with age. Establishing abnormal cutoffs for older babies is an option, but could be difficult. Timing of specimen collection might be critical in selected cases and result in invalid second specimens. Repeat testing of second specimens collected from older newborns should not be considered a reliable specimen for confirmation of all disorders.
- Reporting MS/MS results is the responsibility of each newborn screening program. Screening laboratories should employ a trained, credentialed person to

interpret screening profiles, similar to requirements for MS/MS diagnostic laboratories. What results to report and how to report them should be decided in consultation with state-designated referral centers, health-care practitioners, and public health follow-up staff. Options include

— providing test data for both normal and abnormal results with or without interpretation;

— providing only interpretation (i.e., test results are normal or abnormal); or

— combining these two options and reporting normal results as an interpretation only (e.g., fatty acid oxidation is normal) and reporting abnormal results as observed values with interpretations.

- Program managers should be aware that no U.S. Food and Drug Administration-approved interpretive software for MS/MS newborn screening exists. Thus, individual laboratory validation of interpretive protocols is required.

Specimen and Control Sample Storage

Work Group Proposals

- Stored patient specimens and control samples must be kept frozen at ≤–20 C with humidity ≤30% to ensure long-term storage validity. Desiccants should be used to maintain humidity levels. Laboratories should maintain written policies and procedures that specify storage standards for specimens (*1*).
- When patients' specimens are analyzed after storage, control samples stored under the same conditions should also be used. Results of analyzing stored specimens should be interpreted cautiously because long-term validation studies have not been published.

NEWBORN SCREENING FOLLOW-UP

Workshop discussions regarding patient follow-up were consistent with those of the Newborn Screening Task Force meeting, which was convened by the American Academy of Pediatrics in Washington, D.C. in 1999 (*1*); therefore, proposals regarding follow-up concerns are limited in this report. Newborn screening follow-up includes short- and long-term components. Short-term follow-up tracks patients from a positive test result through diagnosis and acknowledgment by a health-care provider of that diagnosis. Long-term follow-up tracks patients from diagnosis through clinical management and beyond to ensure that they and their family members receive needed services.

Work Group Proposals

- The same short-term follow-up approach should be followed for MS/MS-detectable disorders as for conditions in which delayed treatment poses a high risk of fatal outcomes and for which timely transport of test samples and analysis are essential (e.g., galactosemia and congenital adrenal hyperplasia). For

metabolic disorders in which usual feeding practices might result in acute crisis and risk of death, upon notification of an initial abnormal test result, physicians should advise parents of what short-term feeding measures to take in advance of repeat and confirmatory tests so as to avert a potentially lethal crisis (*33*). In the case of MCAD deficiency, an infant who experiences fasting as the result of loss of appetite is at risk of hypoglycemic crisis, which is preventable if parents ensure that children eat regularly (*34*).

- Although reported false-positive rates are low and metabolic disorders are rare, an increase in the number of diagnosed disorders will require additional follow-up personnel and definitive diagnostic services. Newborn screening program personnel will need technical and resource assistance with developing educational materials and in training staff and health-care providers in follow-up procedures for MS/MS-detectable conditions.
- To conduct adequate long-term follow-up, programs will need to establish or improve patient-tracking systems. Ideally, data management for such a system would include registries to which treatment centers continually provide updated information, treatment compliance, and outcomes.

DIAGNOSIS AND TREATMENT

MS/MS technology can assist in diagnosing metabolic disorders during the newborn period that previously were diagnosed only after symptoms developed. Presymptomatic detection now allows treatment initiation while the infant is healthy and assists in defining the spectrum of clinical disease related to these disorders. MS/MS technology can be used advantageously to screen for selected amino acid disorders for which other newborn screening methods are used. For example, MS/MS can accurately detect elevated phenylalanine levels in dried blood spots taken from infants as young as age $\leq$24 hours. By using the MS/MS-detected phenylalanine/tyrosine ratios, physicians can diagnose PKU earlier and rely on an assay with a reduced false-positive rate (*6*).

For policymakers and program managers, the uncertainty of outcomes from early diagnosis complicates deciding whether to use MS/MS for newborn screening. For example, presymptomatic MCAD-deficiency detection might lead to decreased morbidity and mortality among infants, whereas evidence regarding outcomes for other fatty acid oxidation disorders is lacking (*35*). Because data are still limited, additional research is needed to determine what interventions work for MS/MS-detectable metabolic disorders. Consensus is lacking regarding which disorders should be included in a MS/MS screening panel and for defining a list of mandated tests in the panel. Additional pilot testing is required. Another challenge to the definitive diagnosis of metabolic disease is that persons with one of these disorders might by affected in different degrees of severity (i.e., clinical heterogeneity) with varying physical and biochemical manifestations. Opinions differ as to where to draw the line on the diagnosis of infants as affected or not by a particular disorder. In using MS/MS technology, clinical heterogeneity presents challenges in setting cutoffs to minimize the frequency of false-positive and to prevent false-negative results. The full clinical spectrum of metabolic disorders is unknown because certain MS/MS-detectable disorders are rare and not well-described in the literature. Further, even for classical cases, different mutations in DNA (deoxyribonucleic

acid) can exist. Milder variants exist also, and their natural history is unclear. The term *milder variant* is based on the discovery of a mutation that does not correlate with clinical symptoms and is recognized only when a particular stress is placed on the affected child. Newborns with mild or late-onset variants of metabolic disorders are more likely to be missed by MS/MS. Conclusions based on the outcomes of limited numbers of reported cases are not valid assessments of variants; therefore, prevalence data for variant cases will be sparse until statistically significant numbers of test results are collected and analyzed.

In conjunction with diagnostic questions needing additional research, treatment outcomes are of concern for policymakers and program managers considering MS/MS for newborn screening. Again, long-term studies are needed to evaluate whether outcomes are improved as a result of MS/MS screening and early diagnosis. Clinical treatment and long-term care services are costly; therefore, treatment expense and funding resources for support services are of concern.

Work Group Proposals

- To take advantage of MS/MS technology for detecting abnormal metabolite levels among newborns, many of which are present during the first week of life, rapid transport of blood-spot specimens to the screening laboratory is required. The time from birth to diagnosis should be as short as possible, with an ideal time frame of $\leq$5 days.
- Efforts should be made to reduce handling time within hospitals to decrease time to analysis.
- Optimal specimen transport time from the hospital to the laboratory is $\leq$24 hours, but transport time of $\leq$48 hours is critical. Program managers should consider using courier services and requiring 24-hour delivery of specimens to the laboratory. To ensure delivery, program managers should have contingency plans in place.
- A 6-day work schedule for MS/MS laboratories performing newborn screening is preferable because the first analysis should be performed within a 24-hour turnaround time, with another $\leq$24 hours for retesting and confirmation if test results indicate abnormal levels.
- For all infants suspected of having a metabolic disorder, confirmatory testing, using published standard metabolic testing procedures, should be performed before treatment. Different criteria should be used for diagnostic confirmation testing, but MS/MS technology can be used also.
- Acylcarnitine analysis performed by an MS/MS laboratory is valid for specimens from newborns but might not be for specimens from older infants. Duplicating an analysis by the same or another laboratory adds only limited information, and the results could be misleading. Laboratory results should be correlated with the clinical status. If available, a DNA analysis for common mutations in the disorder would provide further confirmation of the disease.

- Treatment resources might be inadequate as a result of the rarity of metabolic diseases among newborns; therefore, ensuring delivery of clinical services can be a logistical and funding challenge for health-care providers. Access to treatment services that includes specialized care centers, nutritionists, social workers, certified genetics counselors, and specially prepared medically required foods must be ensured before screening is introduced.
- Because of the emotional and financial burden that care, treatment, and outcomes for newborns with MS/MS-detectable metabolic conditions pose on the families, clinicians should communicate concerns and treatment options carefully to parents of possibly affected children (*1,15*).

MS/MS SCREENING EVALUATION

The Newborn Screening Task Force recommends that state and territorial health agencies

- include evaluation, performance monitoring, and quality assurance activities in their newborn screening systems;
- conduct oversight of program operations; and
- monitor and evaluate program performance through collection, assembly, analysis, and reporting of data, including outcome evaluations (*1*).

Specifically, the evaluation of a screening program involves examining the clinical validity (i.e., sensitivity and specificity, positive or negative predictive values), clinical utility (i.e., improvement in health or development outcomes with early treatment), and cost-effectiveness of the screening protocol for each disorder (*36*). Pilot demonstration programs in states could provide information regarding certain variables if they had adequate resources to acquire and report technical and clinical results (*15*). Determination of false-positives, specificity, and positive predictive value is straightforward and can be calculated using a data system that tracks infants from initial test results through diagnosis. Remaining variables require development of more sophisticated and collaborative data collection systems, particularly for evaluating the clinical utility of screening, which for any given disorder, depends on the demonstration that early treatment improves long-term outcomes.

Work Group Proposals

- Long-term storage of leftover specimens is a critical consideration for newborn screening programs. Leftover specimen storage and use should be guided by policies and procedures that include protection against their inappropriate use (*1*). Retention of blood-spot specimens could be pivotal in determining false-negative rates (*1*). False-negatives can be confirmed only by identification of an affected patient clinically or through autopsy findings, and comparing those findings with results obtained by retesting the original blood spot and using storage-control specimens. Correct storage of specimens is required for this process (*15*).

- A key challenge to using MS/MS for expanded newborn screening is the lack of published scientific data regarding MS/MS-detectable disorders among newborns. Specifically, data are needed regarding the results of diagnosis and treatment to justify the expanded screening. Expert opinion regarding the justification for performing expanded screening varies substantially (*1*). A list of the disorders detectable by MS/MS are provided in this report (Table 1). Expert reviewers have concluded that MCAD, one of the disorders that requires MS/MS screening, meets almost all of the criteria to justify newborn screening, and these reviewers recommend collecting additional data through pilot MS/MS screening programs (*35,37,38*). MS/MS also offers certain advantages over traditional methods for detection of PKU and other amino acid disorders (*25,28*).
- Although certain newborn screening programs are expanding without scientific support, program managers should incorporate epidemiologic research methods into implementation efforts so that evaluation results can be used by others facing this challenge.
- To assess the utility of expanded MS/MS screening, national data regarding screening performance and outcomes should be collected. No mechanism beyond the National Newborn Screening and Genetics Resource Center's report is in place for collection of these data. Federal agencies could support the design and implementation of projects targeted for gathering data and retrospectively analyzing the experience of expanded newborn screening programs, but such projects first require development of uniform data reporting protocols. In particular, such projects would require agreement regarding consistent case definitions, including normalization of cutoffs.
- Routine data collection by a single state program is unlikely to be sufficient to evaluate the long-term outcomes of screening for these conditions. Constructing prospective cohorts of patients with rare disorders, although expensive, is one way to address issues of the true incidence and prognosis of these disorders. Prospective cohorts have been constructed for other diseases, notably childhood cancers and hemophilia. Another relevant model for expanded screening research is the multicenter registry of cystic fibrosis patients coordinated and supported by the Cystic Fibrosis Foundation. A federally funded multicenter study could track newborns with positive test results and actively pursue other clinically detected cases. This study would require a substantial long-term funding commitment and would require the expertise and cooperation of epidemiologists and health services researchers in multiple federal agencies.
- Collection of economic data regarding costs and cost savings is essential for analyzing the cost-effectiveness of screening. Collecting economic data requires improved coding techniques for inborn errors of metabolism so that use of health-care services is consistently recorded. Consistent recording would allow financial data collection to justify continuation of programs or third-party–payer reimbursement.

- An epidemiologic perspective should bring additional benefits (e.g., definitions of minimal essential data and improved data coding). Other strategies besides prospective cohort methods are possible. For example, data from medical examiners in different states has led to the realization that some sudden and unexplained childhood deaths can be attributed to specific inborn errors of metabolism with a higher frequency than previously recognized.
- Public health officials and newborn screening program managers evaluate screening systems differently than parents of affected children, primary-care providers, and the public do. Public health officials and program managers focus on positive and negative predictive values and clinical utility. In contrast, the public and physicians without substantial experience with these disorders lack understanding regarding screening and might have unrealistic expectations for treatment outcomes. Both parents and health professionals need to be educated regarding limitations and availability of expanded newborn screening (*1,15*).

CONCLUSION

The goals of the 2000 workshop were to provide guidance for newborn screening program managers and policymakers who are using or planning to use MS/MS technology; workshop goals did not include recommending screening for specific MS/MS-detectable disorders. Interest in using MS/MS technology for newborn screening for an expanded range of inheritable metabolic disorders is increasing throughout the United States. A limited number of public health screening laboratories have introduced MS/MS with minimal difficulty. Others have started MS/MS testing with a limited understanding of MS/MS applications and detectable disorders; those programs have found installation and use of MS/MS instrumentation to be a substantial undertaking. In certain cases, overall system concerns have not received adequate consideration (*1*). The American College of Medical Genetics and the American Society of Human Genetics state that ". . . MS/MS can provide substantial benefit to patients and their families, if thoughtfully integrated into newborn screening programs" (*15*). However, the need to monitor MS/MS screening programs on a collaborative basis, with periodic reappraisal of goals and achievements, is now recognized, and different groups are beginning to work together to better assess concerns, solutions, and outcomes of MS/MS testing.

The overall consensus of the workshop participants is that the public should receive accurate information regarding expanded and comprehensive newborn screening and the evolving knowledge regarding its strengths and weaknesses. State programs that are ensuring universal opportunity, quality control, tracking, and follow-up should continue without interruption. Additional disorders other than metabolic disorders detected by MS/MS are included in comprehensive screening (e.g., congenital adrenal hyperplasia, cystic fibrosis, sickle cell disease, and biotinidase deficiency). Expansion of screening should include the more common disorders that are available in certain programs (*1*).

Work Group Proposals

As a result of information shared at the 2000 workshop and efforts of the work group, the following overall proposals are offered:

State and territorial health agencies should

- consider using MS/MS and other expanded newborn screening technologies and actively participate in future workshops because these agencies are the direct sources of funding and regulations for prevention efforts. As research and reported data grow regarding MS/MS for newborn screening, public health agencies will want to access this technology either directly or through regional agreements. Staying current with technological developments will be critical for policymakers and program managers.
- anticipate difficulties in implementing MS/MS testing because the technology requires new, expanded, and expensive resources. Those resources include
  - — investments in equipment;
  - — expanded information technology support for interpretation, reporting, tracking, and outcome evaluation;
  - — staff training in clinical and laboratory aspects of detectable disorders;
  - — access to reference laboratories for confirmation of diagnosis; and
  - — assurance of access to adequately skilled clinicians for treatment and counseling.
- involve health-care practitioners, laboratory directors, birthing hospitals, parents, lay advocacy groups, and third-party payers in a collaborative effort to plan and define the state and territorial programs and obtain the legal authority and funding necessary for implementation.
- consider contracting with other state laboratories, private laboratories, or academic medical centers for laboratory services that are too expensive for local program resources. Alternatively, information could be provided to health-care practitioners, hospitals, and parents explaining the options for supplemental testing services, and state/territorial program staff could facilitate access to these services. States that contract for laboratory or other services should retain the functions of quality assurance and monitoring (e.g., speed and accuracy of testing and reporting, tracking, and ensuring quality clinical follow-up).

Federal health agencies should

- provide leadership and support to assist states and territories in implementing MS/MS technology. An effective model for federal involvement in newborn screening is the assistance that was provided to states and territories when implementing sickle cell disease testing. A 1986 National Institutes of Health consensus development conference (*39*) recommended sickle cell disease screening for newborns. Congress then appropriated $8 million for the Special Projects of Regional and National Significance program administered by HRSA. Using that funding, HRSA awarded grants to enhance the screening infrastructure. States then reviewed the scientific information, appropriated funding, and added sickle cell disease to their newborn screening programs. CDC, in cooperation with HRSA, developed a quality-assurance program and funded studies for effectiveness evaluation of sickle cell screening methods and programs.

- support a national MS/MS screening work group with three subcommittees,
  - — a laboratory methods group to address standards, quality assurance, and methodologic improvements;
  - — a clinical group to define the detectable disorders, available interventions, and requirements for metabolic diagnosis and management; and
  - — an epidemiologic group to design and implement an evaluation effort that would include collecting data regarding effectiveness of different screening policies, disorder prevalence and trends, long-term outcomes, and cost-effectiveness and cost-benefit.

  Representatives from the American Academy of Pediatrics, American College of Medical Genetics, American Public Health Association, Association of Public Health Laboratories, Association of State and Territorial Health Officers, National Newborn Screening and Genetics Resource Center, CDC, and HRSA should actively participate in workshop, work group, and subcommittee activities.
- sponsor long-term epidemiologic studies of MS/MS screening to document the natural history of metabolic diseases, establish data collection protocols, and evaluate MS/MS technology's impact.
- provide resources to support state and local staff training in MS/MS analytic techniques and efforts to provide analytical standards and proficiency testing.
- provide fiscal and technical support for long-term follow-up, large-scale data sharing, or development of laboratory quality-assurance programs to prevent MS/MS applications of mixed quality and effectiveness resulting from independent and isolated actions.

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Appendix

Synopses of Selected Papers Presented at the Tandem Mass Spectrometry for Metabolic Disease Screening Among Newborns Workshop, San Antonio, Texas, June 2000

Scientists from all operating tandem mass spectrometry (MS/MS) newborn screening laboratories and programs in the United States — public health, academic, and private — and two international experts (Rodney J. Pollitt, Ph.D., United Kingdom and Bridget Wilcken, M.B., Ch.B., Australia) were invited as discussants for the workshop held in June 2000. The discussant group included the pioneers of MS/MS applications in newborn screening, Charles R. Roe, M.D.; Donald H. Chace, Ph.D.; David S. Millington, Ph.D.; and Edwin W. Naylor, Ph.D., as well as scientists from the two companies that manufacture MS/MS equipment used for newborn screening. The workshop was a joint effort that involved cooperation among public health agencies, academic institutions, and the private sector with shared interest in enhancing the use and quality of MS/MS technology. Approximately 50 observers from newborn screening programs in the United States with future expectations for using MS/MS also attended the workshop.

Each invited discussant was asked to provide a 1-page summary relating their experiences with MS/MS for any of the applicable parameters (e.g., setting-up and operating MS/MS, selection of cutoffs, associated follow-up of infants with identified disorders, medical confirmation of disorders, and treatment of disorders). Summaries were submitted of presentations given at the plenary session and breakout group meetings. Invited discussants were also asked to provide a list of what they believed were the 5–10 most important questions, in a priority order, regarding the application and needs for MS/MS in screening, confirmation, quality assurance, follow-up, and treatment of metabolic disorders among newborns. These presentations, summaries, and lists of questions were used to guide breakout groups in their deliberations and discussions for the development of work group proposals for MS/MS in newborn screening. Selected synopses of summary papers that represent the context of the workshop are presented here.

SELECTED PRESENTATIONS REGARDING LABORATORY PRACTICE

Testing Newborn Specimens by Tandem Mass Spectrometry: The First 16 Months' Experience in the New England Program — Thomas H. Zytkovicz, Ph.D.; Donna Johnson; Denise Rojas, M.P.H.; Eileen Fitzgerald; *New England Newborn Screening Program, Jamaica Plain, Massachusetts*

On February 1, 1999, the New England Newborn Screening Program began using tandem mass spectrometry (MS/MS) to test newborns for 23 disorders, including 9 amino acid disorders, 7 organic aciduria disorders, and 7 fatty acid oxidation disorders. However, before using MS/MS, we validated the experimental method.* MS/MS instrument

*__Source__: Rashed MS, Bucknall MP, Little D, et al. Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. Clin Chem 1997;43:1129–41.

bias was determined by comparing test results with preexisting analytical methods (e.g., high-pressure liquid chromatography and bacterial inhibition assay). Blood controls were prepared by fortifying samples with amino acids and acylcarnitines and sending these samples to other laboratories for MS/MS analysis of the markers. These interlaboratory results were used to compare our laboratory's performance with that of established laboratories. Approximately 170,400 newborns were tested for phenylketonuria, maple syrup urine disease, and homocystinuria. MS/MS identified six infants with phenylketonuria, nine with hyperphenylalaninemia, one with maple syrup urine disease, and three infants with homocystinuria/hypermethionemia. Approximately 100,000 newborns were tested for 20 other disorders. MS/MS tentatively identified seven infants with medium-chain acyl-CoA dehydrogenase; four with short-chain acyl-CoA dehydrogenase; two with phenolic acid; and one each with methylcrotonyl-CoA carboxylase; carnitine palmitoyl transferase, type II; and very-long–chain acyl-CoA dehydrogenase. The majority of these infants are undergoing DNA (deoxyribonucleic acid), urine, or in vitro testing to confirm MS/MS diagnoses.

Comprehensive, High-Quality Analytical Approach to Newborn Screening Using Tandem Mass Spectrometry — Donald H. Chace, Ph.D.; *Neo Gen Screening, Inc., Bridgeville, Pennsylvania*

Because MS/MS technology can be used for screening multiple disorders in a single analysis, low-prevalence disorders can be included with higher prevalence analytes without a substantial additional cost. However, MS/MS screening results require interpretation by experienced metabolic specialists and confirmation by other analytical or diagnostic techniques. MS/MS uses stable isotope internal standards as an essential component of the method, and therefore, it is precise and accurate in controlled tests. Nevertheless, blood volume from filter paper blood-spot specimens can be inaccurate and reduce the reproducibility of MS/MS that is observed in analyses of liquid specimens (e.g., serum or plasma). Ratios of $\geq$2 components improve diagnostic accuracy and reproducibility that otherwise would be lost by using filter paper blood-spot specimens. As this emerging technology develops, sample preparation, analyses, data processing, and interpretations will be modified. New assays will expand the current panel of MS/MS-detectable disorders or provide a source for confirmation. Further, as genetic testing expands to include additional molecular approaches, complementation of these methods will provide comprehensive analyses that will better serve public health needs.

Introduction of Tandem Mass Spectrometry Into the Newborn Screening Environment — Michael R. Morris, Ph.D.; *Micromass United Kingdom Ltd., Manchester, United Kingdom*

Personnel training and laboratory support can resolve certain problems related to introducing tandem mass spectrometry for newborn screening, but program managers must develop their own experiences before determining that MS/MS is a stable tool to be used in routine newborn screening. Although a detailed knowledge of physics and instrument operation is unnecessary, program managers and laboratorians should understand basic operational details. High-throughput screening of extracted blood samples is

an exacting test, and certain critical factors must be considered to maintain optimum instrument performance, including the following:

- **Sample preparation.** If samples are prepared in a substandard manner, the validity of the assay becomes questionable. Addition of internal standard materials at the beginning of the extraction procedure allows monitoring of the extraction and derivatization as well as instrument performance. Quality of solvents, standards, and other materials used in sample preparation procedures must be assessed for suitability.
- **Instrument sensitivity.** Using a benchmark solution to visually check instrument performance at the start of a day's work can identify problems in a timely manner. Injection of a standard sample that can be monitored in real time can give the experienced user information regarding the status of the flow path and injection system, the cleanliness of the ion source, and the status of the mass calibration. Users become adept at rapidly drawing conclusions on the basis of the arrival time of the sample after injection, the location of the individual peaks on the mass scale, the instrument resolution, and the absolute and relative intensities of the peaks.
- **Sample inlet system blockages.** Investigative samples contain a substantial number of organic-soluble compounds and have the potential to introduce particulate matter. A logical assessment of the liquid-flow path and regular monitoring of the back-pressure of the liquid chromatography pump will increase efficiency.
- **Use of operational qualification and quality-control samples.** Ideally, quality-control samples should be used to monitor the performance of the assay for all compounds of interest. Practically, monitoring an individual compound from each class of analytes being measured might be sufficient to prove analytical effectiveness. Also, tests should be run to ensure that particular internal standards are present at the correct concentration.

Laboratories that have played key roles in introducing MS/MS into newborn screening have developed cross-checks to monitor assay performance and provide analytical safeguards. In addition, interlaboratory communication that focuses on emerging problems and possible solutions is strongly recommended.

Selection of Reporting Cutoffs in Newborn Screening: Patient-to-Normal Ratio —
Joerg N. Pirl, Ph.D.; Rong Shao, M.D.; Michael Petros, M.S., M.P.H.; *Illinois Department of Public Health, Chicago, Illinois*

The selection process for reporting cutoffs is based on statistical evaluations of historical patient data, which might change as methods and procedures are updated, thus producing a substantial number of false-positive results. Because test results are method-dependent, reference ranges or normal values are needed for interpretation. If the patient results are expressed relative to that normal value, the resulting patient-to-normal ratio (PNR) is a unitless number that is a measure of the deviation of the patient result from that of the normal population. When normal values are calculated from

within-run data, uniform variations associated with matrix, calibration, recovery, accuracy, and instrument will cancel. In a newborn screening laboratory, approximately 95% of samples received are from patients with normal levels, and PNRs derived from patients analyzed under approximately identical circumstances are method-independent and, thus, interlaboratory comparable.*

We compared rapid-flow analysis (RFA) and MS/MS phenylalanine (phe) results from approximately 14,000 neonatal dried blood spots. Included were 267 patients with levels above our RFA cutoff of 4.0 mg/dL and 45 patients with confirmed phenylketonuria or hyperphenylalaninemia. For newborns with normal levels, concentrations of phe were usually distributed around the mean. When the results were arranged in ascending order, the result of the median patient agreed closely with the mean of the median 68% (mean ± 1 standard deviation [SD]) and 95% (mean ± 2 SD) of the population. This agreement was true also for ratios of phe to selected other amino acids. The median deviated by <1% from the 68% and 95% population mean, and the concentration of phe as well as the ratio of phe to the other amino acids was linearly related to the median 60% of the normal patients with a slope near zero; for phe, the slope was 0.00087 mg/dL/patient. That is, when results were sorted numerically, this group of patients had the same or nearly the same results. For any given routine run (i.e., MS/MS or RFA, with a minimum of one 96-well microplate) the result of the median patient (patients arranged in ascending order) differed <3% from the mean of the central 68% and <5% from the central 95% mean. MS/MS, in light of its high analyte specificity, produced a lower phe concentration than RFA but did not substantially improve the predictability for disease-correlation coefficient, MS/MS versus RFA >0.9. RFA positive predictive value was 18.2%. To examine internal metabolite ratios and to minimize effects from specimen quality or feeding, in addition to phe, we also analyzed these patients by MS/MS for leucine (leu), methionine (met), tyrosine (tyr), alanine (ala), valine (val), and serine (ser).

PNR was calculated for phe, phe/leu, phe/met, phe/tyr, phe/ala, phe/val, and phe/ser by dividing the results of the patients by the median result for that parameter, with results sorted numerically. The final output presented the PNR values for phe and those of the internal metabolic ratios in tabular form. PNR-phe/tyr of <2.0 was associated with normalcy. The lowest PNR-phe/tyr in hyperphenylalaninemia patients was 2.36 and 4.62 in cases of classic phenylketonuria. The positive predictive value was 83.8% if PNR for all internal ratios was considered and 38.4% on the basis of PNR for phe/tyr only. The negative predictive value was 100% in all cases.

Implementation of Tandem Mass Spectrometry in Wisconsin's Newborn Screening Program — Gary L. Hoffman; Thomas Litsheim; Ronald H. Laessig, Ph.D.; *Wisconsin State Laboratory of Hygiene, Madison, Wisconsin*

After we acquired a tandem mass spectrometry (MS/MS) instrument to measure acylcarnitines in dried blood specimens, we devoted approximately 1 month to learning its operation. This learning period consisted of a) one, 2-day session with the service engineer during the setup reviewing instrument calibration and routine maintenance requirements; b) two, 3-day sessions with instrument manufacturer's application specialists to establish sample preparation, method calibration, and data reduction; c) 1 week independently developing a comfort level with instrument operation; and

*__Source__: Hubbard AR, Margetts SML, Barrowcliffe TW. International normalized ratio determination using calibrated reference plasmas. Br J Haematol 1997;98:74–8.

d) 1 week at a training course at the manufacturer's facility. This training enabled us to begin pilot testing.

One of the goals of the pilot testing phase was to collect acylcarnitine data to establish reporting profiles for fatty acid oxidation and organic aciduria disorders. The first step was a literature review of the acylcarnitines associated with each of the 14 fatty acid oxidation and organic aciduria disorders mandated by the Wisconsin Department of Health's Newborn Screening Advisory Committee. Although published literature does not agree on the acylcarnitine profile for each disorder, we established a composite profile for each one. After collecting 5,000 random observations for each acylcarnitine, we established an abnormal reporting level at the mean concentrations plus four standard deviations. We further adjusted these abnormal reporting levels as follows: a) comparing them against those already published by other laboratories using MS/MS to identify newborns with these 14 metabolic disorders, and b) consulting with an experienced metabolic expert in the clinical diagnosis of these disorders. For the majority of acylcarnitines, the four standard deviation level was maintained, although certain adjustments were made that increased the cutoff of some acylcarnitines to $\geq$5 standard deviations from the mean. In addition to acylcarnitine levels, we added multiple concentration ratio-based criteria to the abnormal profiles for certain disorders. Using this preliminary criteria, we referred 13 babies for confirmatory testing from the approximately 50,000 specimens screened during the pilot study. Five (i.e., four medium-chain acyl-CoA dehydrogenase and one short-chain acyl-CoA dehydrogenase disorders) were confirmed.

A secondary goal of the pilot study was to determine how the MS/MS technology could be incorporated into the routine newborn screening operation. A primary focus was on the ability of the MS/MS instrumentation to process the specimens in a timely manner (i.e., $\leq$24 hours after receipt) so delay in reporting all results would be minimal. With modifications, we reduced sample preparation time to <10 minutes after punching* the sample from the blood-spot specimen. Because the instruments' throughput is 1 sample/1.5 minutes and it can operate overnight unattended, the system could handle 500 samples/day. On the basis of the success of the pilot testing phase, which established abnormal reporting levels and instrument reliability, we began routine testing for 14 metabolic disorders by MS/MS in April 2000.

**Punching* is a procedure that takes an aliquot portion (e.g., 1/8 inch) from the dried blood spot contained on the filter paper.

SELECTED PRESENTATIONS REGARDING NEWBORN SCREENING FOLLOW-UP

Incidence and Follow-Up Evaluation of Metabolic Disorders Detected by Newborn Screening in North Carolina Using Tandem Mass Spectrometry — Joseph Muenzer, M.D.; Dianne M. Frazier, Ph.D.; Shawn E. McCandless, M.D.; *University of North Carolina, Chapel Hill, North Carolina*; Elizabeth G. Moore, M.S.W.; Susan D. Weavil; Shu H. Chaing, Ph.D.; *Department of Health and Human Services, Raleigh, North Carolina*; David S. Millington, Ph.D.; *Duke University Medical Center, Research Triangle Park, North Carolina*

Since August 1997, the North Carolina Newborn Screening Program has been screening all newborn infants by using tandem mass spectrometry (MS/MS) for selected amino acid, organic acid, and fatty acid oxidation disorders. Initially, a statewide pilot study lasting 20 months was conducted, and on the basis of that pilot study, the North Carolina Newborn Screening Program incorporated MS/MS screening into the state newborn screening panel. During the pilot study, Neo Gen Screening, Inc., (Bridgeville, Pennsylvania) screened 194,384 newborns (214,634 specimens) and identified 259 (0.13%) samples as abnormal. Metabolic disorders were confirmed for 31 infants; results indicated that 14 children had medium-chain acyl-CoA dehydrogenase deficiency; 1, long-chain fatty acid oxidation disorder; 6, hyperphenylalaninemia; 1, hypermethionemia; 3, citrullinemia; 1, argininosuccinic aciduria; and 5, organic aciduria disorders.

During a subsequent phase of implementation (April 1999–June 2000), the North Carolina Newborn Screening Laboratory screened 131,776 newborns (147,286 specimens). Initial cutoffs resulted in false-positive detection rates of >1.9%, but revised cutoffs resulted in a <0.85% false-positive rate. Metabolic disorders were confirmed for 27 infants; results indicated that 10 children had medium-chain acyl-CoA dehydrogenase deficiency; 1, short-chain acyl-CoA dehydrogenase deficiency; 9, hyperphenylalaninemia; and 7, organic aciduria disorders. Since MS/MS screening began in North Carolina, disorders among three infants (one with late-onset methylmalonic acidemia and two with glutaric acidemia, type I) were missed by MS/MS screening, but the disorders were diagnosed clinically before age 1 year for all three infants.

Patient follow-up in North Carolina is coordinated by the Division of Genetics and Metabolism, Department of Pediatrics, University of North Carolina at Chapel Hill (UNC). A two-tier follow-up approach has been used with abnormal MS/MS screening results. When the MS/MS results indicate an inborn error of metabolism, the screening laboratory notifies UNC by telephone and facsimile. UNC staff then contact the local health-care provider by using information provided on the newborn screening card. Follow-up evaluation and recommendations for additional testing, on the basis of the MS/MS laboratory findings, are made by UNC personnel so the suspected diagnosis can be confirmed, the family counseled, and treatment initiated. Initial MS/MS screening results that are borderline (i.e., a possible inborn error of metabolism) require repeat testing. If a second specimen indicates abnormal levels, UNC staff coordinate additional testing (i.e., for abnormal amino acids, a plasma amino acid analysis is run; for an abnormal acylcarnitine profile, plasma acylcarnitine profile and urinary organic acid analyses are run).

All infants in North Carolina with medium-chain acyl-CoA dehydrogenase deficiency detected by newborn screening are treated with carnitine and are maintained on breast milk or infant formula with no fat restriction to age 1 year. Parents are instructed to avoid

prolonged fasting and to use a glucometer to monitor blood glucose levels when they are concerned regarding the child's oral intake or clinical status. No deaths, significant hypoglycemic events, or seizures have occurred among the infants with medium-chain acyl-CoA dehydrogenase deficiency identified by newborn screening. Screening by MS/MS, with careful follow-up, can prevent the majority of deaths and serious sequelae of medium-chain acyl-CoA dehydrogenase deficiency during the first years of life. However, MS/MS is not entirely specific for this error of metabolism and detection of abnormal but nondiagnostic metabolites can occur. Since MS/MS analysis began in the North Carolina Newborn Screening Laboratory, borderline results have been common with propionyl-acylcarnitine, 3-hydroxy-isovalerylcarnitine (C5-OH), and tyrosine. Adjustment of cutoff levels has reduced the false-positive rate, but additional adjustment might be needed on the basis of follow-up evaluation of the borderline results. Close coordination between the MS/MS screening laboratory and the metabolic clinic/biochemical geneticists is needed to determine screening parameters, adjust the cutoff levels to reduce false-positive and false-negative results, and facilitate clinical follow-up.

Notification Experience with Newborn Disorders Detected by Tandem Mass Spectrometry in the Early Experience of the New England Program — George F. Grady, M.D.; Thomas H. Zytkovicz, Ph.D.; Deborah Marsden, M.D.; Cecilia Larson, M.D.; Vivian Shih, M.D.; *New England Newborn Screening Program, Jamaica Plain, Massachusetts*

A series of protocols has emerged for notifying pediatricians when a newborn under their care is identified by tandem mass spectrometry (MS/MS) screening as having an out-of-range amino acid or acylcarnitine value. For the amino acids, phenylalanine (for phenylketonuria [PKU] or hyperphenylalaninemia [HPA]), leucine plus isoleucine (for maple syrup urine disease [MSUD]), and methionine (for homocystinuria [HCU]), the MS/MS analysis was performed for approximately 171,500 newborns. The net yield of confirmed (repeatedly positive) cases was similar to that during the prior era of bacterial inhibition assay (i.e., six PKU plus nine HPA, one MSUD, and one HCU plus two hypermethionemia). Persistence of biological confounders affected clinical interpretation of the phenylalanine, leucine, or methionine for 102 newborns, who required repeat specimens because of immaturity or systemic illness.

For approximately 109,400 newborns, the screening profile by MS/MS included separate recordings of tyrosine and the basic amino acids, ornithine, citrulline, and arginine. Testing repeat specimens from 24 infants with an initial elevation of tyrosine, and from 24 infants who had initial elevations of ornithine, citrulline, or arginine, yielded no confirmed cases of specific disorders that would have explained the elevations. Nevertheless, when physicians associated with our screening program staff contacted the infants' physicians regarding the elevation, our staff provided information regarding potential signs and symptoms and possible future interventions.

The 109,400 newborns were also screened by MS/MS for certain acylcarnitines that had side chains containing $\leq$18 carbons (e.g., C18), to detect fatty acid oxidation and organic aciduria disorders. For an additional 10,000 infants, only the medium-chain acylcarnitines were measured. Among those infants whose test results indicated fatty acid oxidation disorders, the medium-chain candidate group included 36 infants of whom a) two had an initial C8 of 9–10 µM (the parents were referred immediately to metabolic clinics); b) five had an initial C8 of 1.8–3.2 µM (parents were given the option of direct

referral or awaiting results of C8 on a second specimen before deoxyribonucleic acid or urinary acylglycine analysis); and c) 29 had an initial C8 elevation of 0.5–0.8 µM. The cases in group a were homozygous for the A985G marker; group b was incompletely analyzed but includes ones with positive urinary samples or a single copy of A985G; and group c revealed no elevated C8 on repeat testing and the deoxyribonucleic acid assay was left to personal choice. Upon initial notification, all pediatricians were asked to verify feeding status of the infant and to advise parents to avoid gaps in feeding.

Fatty acid oxidation disorder detections also included four presumptive cases of short-chain acyl-CoA dehydrogenase deficiency that had initially elevated butyrylcarnitine, C4 (range: 2.6–3.7 µM), persisting on a repeat specimen or accompanied by the respective urine acylglycine, deoxyribonucleic acid, or in vitro markers. In 19 other newborns, initial elevations of C4 (>1.9 µM) did not persist (the notification protocol was analogous to the medium-chain protocol). The remaining four notifications included two with an initial elevation of C16, one of which was a fatal case of carnitine palmitoyl transferase, type 2; and two with elevated C14:1, one of which was confirmed as very long-chain acyl-CoA dehydrogenase deficiency.

Organic aciduria disorder detections included two propionic acidemias (among 23 notifications when propionylcarnitine, C3, was >8 µM) and one beta-methylcrotonyl CoA carboxylase deficiency (among 12 notifications of C5 OH >0.8 µM). Because early onset central nervous system problems were a greater concern for this disorder than for fatty acid oxidation disorder, notification protocols were more aggressive in spite of lower specificity of the initial elevated marker.

Newborn Screening for Medium-Chain Acyl-CoA Dehydrogenase Deficiency —
Sophia S. Wang, Ph.D.; *National Center for Environmental Health, CDC, Atlanta, Georgia*

Increasingly, programs are screening for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency among newborns, which has prompted implementation of tandem mass spectrometry (MS/MS) technology for such testing. If newborn screening programs conduct systematic follow-up and collect data beyond diagnosis, they can serve as models to be used in evaluating programs, determining clinical validity and utility, and providing necessary population-based data. To ensure and evaluate current programs, the Institute of Medicine's core functions of public health (i.e., assessment, policy development, and assurance and evaluation) should serve as the evaluation framework.* Program assurance and evaluation requires the collection of key follow-up data beyond the newborn period to track receipt of services and prevention of adverse outcomes. Essential data include short- and long-term process and outcome measures.† Key short-term measures include the percentage of live-born infants adequately screened and the timeliness of diagnoses and treatment; essential long-term measures include assessment of adverse health outcomes beyond the newborn period. These data are also essential for developing future programs. Collection of follow-up data also facilitates

*__Source__: Institute of Medicine. Future of public health. Washington, DC: National Academy Press, 1988.
†__Source__: Gordis L. Using epidemiology to evaluate health services. In: Gordis L. Epidemiology. 2nd ed. Philadelphia: WB Saunders Co., 1996:217–28.

assessment of key test parameters (i.e., clinical validity and utility),* which provide information regarding testing accuracy and utility. Clinical validity is defined as how well the test predicts the phenotype; clinical utility measures the benefits and risks of early detection for those persons among whom disease is detected. Lastly, systematic collection of key follow-up parameters can provide needed population-based data regarding these rare disorders. Data regarding incidence, prevalence, and clinical outcomes can be determined for the populations being tested and contribute to the elucidation of the natural history of MCAD deficiency. Furthermore, the penetrance of the mutations associated with MCAD deficiency can be investigated. On the basis of rates of heterozygosity and under Hardy-Weinberg conditions, more MCAD deficiency cases are expected than are currently observed, leading to a substantial number of infants with asymptomatic MCAD deficiency and uncertainty as to who will manifest symptoms and who will remain asymptomatic.† Although diagnosing MCAD deficiency among children is the primary goal of current newborn screening programs, including systematic collection of key follow-up data is vital to ensuring optimal functioning and utility of programs.

Grouping Metabolic Disorders Detected by Tandem Mass Spectrometry to Maximize Political Impact on Legislators and Policymakers — William J. Rhead, M.D., Ph.D.; *Medical College of Wisconsin, Elm Grove, Wisconsin*

Effectively presenting tandem mass spectrometry (MS/MS) and universal newborn screening to legislators and policymakers is an essential preliminary step that differs from actually implementing expanded programs. When seeking legislative or administrative approval at the state or territorial level, presenting the screened disorders to maximize their comprehensibility for persons without scientific or medical backgrounds is useful. Thus, medium-chain acyl-CoA dehydrogenase disorder, long-chain hydroxy acyl-CoA dehydrogenase deficiency, and related hypoglycemic disorders could be grouped and presented under a "low blood sugar" or "sudden infant death syndrome-like" category. Disorders producing encephalopathy (e.g., hyperammonemias and maple syrup urine disease) could be grouped together in a "coma" category; and 3-methylcrotonyl carboxylase deficiency and propionic, methylmalonic, and isovaleric acidemias could be grouped under a "ketoacidotic/acidemic/acid blood" category. These categories are examples and alternative assemblages or designations can be created to maximize their political impact. Grouping rare disorders with unpronounceable names into categories with comprehensible names can accelerate policymakers' understanding and implementation of MS/MS technology for newborn screening programs.

*__Source:__ Institute of Medicine/Committee for the Study of the Future of Public Health. Promoting safe and effective genetic testing in the United States: final report of the Task Force on Genetic Testing. Holtzman NA, Watson MS, eds. Washington, DC: National Academy Press, 1998:1–180.

†__Source:__ Wang SS, Fernhoff PM, Hannon WH, Khoury MJ. Medium chain acyl Co-A dehydrogenase deficiency human genome epidemiology review. Genetics in Medicine 1999;1:332–9.

SELECTED PRESENTATIONS REGARDING DIAGNOSIS AND TREATMENT

Supplemental Neonatal Screening from Acylcarnitine Analysis Using Tandem Mass Spectrometry: Reliability and Specificity — Charles R. Roe, M.D.; *Baylor University Medical Center, Dallas, Texas*

The majority of the disorders detectable by tandem mass spectrometry (MS/MS) are characterized by multiple clinical phenotypes.* This phenotypic complexity is compounded by acylcarnitine profiles, which are not always specific for a single disease. Diseases that are associated with identical or overlapping acylcarnitine profiles include the following examples:

- Long-chain hydroxy acylcarnitine deficiency is indistinguishable from trifunctional protein deficiency.
- Carnitine palmitoyltransferase II is indistinguishable from carnitine acylcarnitine translocase deficiencies.
- The observation of an increase in 3-hydroxy-isovaleryl-carnitine raises the possibilities of 3-methylcrotonyl-CoA carboxylase, 3-methylglutaconic, hydroxymethylglutaryl-CoA lyase, and multiple carboxylase deficiencies.
- An increase in an acylcarnitine containing five carbons can indicate either isovaleric acidemia or the recently identified S-2-methylbutyryl-CoA dehydrogenase deficiency.

Only three disorders exist for which the acylcarnitine profile is completely disease-specific: medium-chain acyl-CoA dehydrogenase deficiency, glutaric aciduria type I, and malonic aciduria. Distinctions among diseases that have similar acylcarnitine profiles can be aided by knowledge of the patient's clinical course, but that information might not be available for interpreting abnormal test results before symptom onset. Recent descriptions of inherited disorders that have the same blood-spot acylcarnitine profile† emphasize the need for additional documentation to accurately diagnose the specific disorder and subsequently manage treatment appropriately. Similarly, in vitro demonstrations have been reported of distinct acylcarnitine profiles for different clinical phenotypes of the same disease, even with the same mutation.§ Acylcarnitine profiles from newborn blood spots do not correlate with these clinical phenotypes. Finally, not all diseases that are potentially detectable by MS/MS acylcarnitine analysis have been observed among newborns.

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*__Source__: Roe CR, Ding JH. Disorders of mitochondrial function. In: Scriver CR, Beaudet AL, Sly WS, eds. Metabolic and molecular bases of inherited diseases. New York: McGraw-Hill. 2001.

†__Sources__: Roe CR, Cederbaum SD, Roe DS, Mardach R, Galindo A, Sweetman L. Isolated isobutyryl-CoA dehydrogenase deficiency: an unrecognized defect in human valine metabolism. Mol Genet Metab 1998;65:264–71; and Gibson KM, Burlingame TG, Hogema B, et al. 2-methylbutyryl-coenzyme A dehydrogenase deficiency: a new inborn error of L-isoleucine metabolism. Pediatr Res 2000;47:830–3.

§__Sources__: Roe CR, Roe DS. Recent developments in the investigation of inherited metabolic disorders using cultured human cells. Mol Genet Metab 1999;68:243–57; and Roe CR, Roe DS. Detection of gene defects in branched-chain amino acid metabolism by tandem mass spectrometry of carnitine esters produced by cultured fibroblasts. In: Harris RA, Sokatch JR, eds. Methods in enzymology. Vol 324. San Diego, California: Academic Press, 2000:424–31.

Tandem Mass Spectrometry in the New South Wales Newborn Screening Program — Bridget Wilcken, M.B., Ch.B.; Veronica Wiley, Ph.D.; *New Children's Hospital, Sydney, Australia*

We introduced electrospray tandem mass spectrometry (MS/MS) into the New South Wales newborn screening program in 1998.* We have screened 196,000 babies, usually on day 3 of life. A total of 50 babies had confirmed defects, approximately the expected rate based on 20 years' previous experience in our statewide biochemical genetics laboratory or on known mutation frequencies: phenylketonuria 28 (21 expected); biopterin defects, two; other aminoacidopathies, five; organic aciduria disorder, three; medium-chain acyl-CoA dehydrogenase deficiency, six (six expected); other fatty acid oxidation disorders, two; noninborn errors (neonatal hepatitis or maternal B12 deficiency), four. Three were known false-negatives, cases of nonketotic hyerglycinaemia, tyrosinaemia type I, and cobalamin C defect. Only 210 repeat samples were requested from babies not having a proven defect. The substantial number of primary analytes used gives a risk for an unacceptable recall rate. Analysis of many retrospective newborn samples from cases later diagnosed clinically is required. An experienced biochemical genetics laboratory and quality-assurance programs are requirements for diagnosis and later monitoring. Where the screening laboratory is not linked to a biochemical genetics laboratory, record keeping will be more difficult because final diagnosis takes time, and the risk exists of recording cases that are really false-positives. Because diagnosis of clinically nonsignificant conditions can be a problem where experienced teams are not available, a limited number of specified disorders should be sought in the first instance. Treatment of asymptomatic babies has not proven to be a problem and is not different from the familiar phenylketonuria situation. Certain potentially detectable disorders are extremely rare. Follow-up should be done only by a clinical biochemical geneticist experienced in managing metabolic disorders, with multidisciplinary care, expert dietician, and biochemical genetics laboratory support. Evaluation of cost-benefit and reduction in morbidity and mortality will be extremely difficult unless accurate, comprehensive records are available for the state or area. We have records of all relevant inborn errors of metabolism detected in New South Wales over the last 20 years. We will use historical controls to come to an estimate of benefit. Randomized controlled trials for the most part will not be possible because of the rarity of individual conditions.

University of Wisconsin Biochemical Genetics Program — Jon Wolff, M.D.; Sandra van Calcar, M.S.; Kristine K. Hanson, M.S.; *University of Wisconsin, Madison, Wisconsin*

In June 1999, the University of Wisconsin Biochemical Genetics Program became involved in the follow-up of abnormal acylcarnitine test results. Our team includes a board-certified biochemical geneticist, a nutritionist, and a genetics counselor. The state laboratory reports all abnormal test results to us and the physician of record. Immediately, our geneticist contacts the child's physician to discuss the diagnosis and arrange follow-up testing. For all disorders, follow-up testing includes urinary organic acids and repeat acylcarnitines, which usually takes 1–2 weeks. During this time, the physician is asked to watch for signs of metabolic crisis and instructed how to treat a patient having

*__Source__: Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass-spectrometry: 12 months' experience in NSW Australia. Acta Paediatr Suppl 1999:432:48–51.

an acute episode (i.e., with intravenous fluids or glucose). The genetic counselor identifies or creates resource materials for the physician and family and is available to the family during this time. If an abnormal test result is confirmed by follow-up testing, the child is examined and the parents provided nutrition consultation and genetic counseling. Our patient treatment for confirmed short- and medium-chain acyl-CoA dehydrogenase disorders includes four components,

- avoidance of fasting;
- administration of carnitine at 50 mg/kg/day, divided, 3 times/day;
- consumption of a moderately low-fat diet (e.g., 30% kcal from fat); and
- prompt treatment for vomiting episodes with intravenous fluids or glucose.

An emergency protocol is written for each child and given to parents for delivery to emergency department personnel, if needed. Because increasing physician awareness and creating resource materials for physicians and patients' families is a high priority, we are developing an education module for physicians.

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