Tandem mass spectrometry (MS/MS) has been used for several years to identify and measure carnitine esters in blood and urine of children suspected of having inborn errors of metabolism. Indeed, acylcarnitine analysis is a better diagnostic test for disorders of fatty acid oxidation than organic acid analysis because it can often detect these conditions when the patient is not acutely ill. More recently, MS/MS has been used in pilot programs to screen newborns for these conditions and for disorders of amino and organic acid metabolism as well. The purpose of this article is to describe MS/MS and discuss its potential role in newborn screening programs.

The modern tandem mass spectrometer usually consists of two quadrupole mass spectrometers separated by a reaction chamber or collision cell; the latter is often another quadrupole. The mixture to be analyzed is subjected to a soft ionization procedure (e.g., fast atom bombardment or electrospray) to create quasimolecular ions, and is injected into the first quadrupole, which separates these parent ions from each other. These ions then pass (in order of m/z ratio) into the reaction chamber, where they are fragmented; the m/z ratios of the fragments are then analyzed in the second quadrupole. Because separation of compounds in the mixture is by mass spectrometry instead of chromatography, the entire process, from ionization and sample injection to data acquisition by computer, takes only seconds.

The computer data can be analyzed in several ways. One can use a parent ion mode to obtain an array of all parent ions that fragment to produce a particular daughter ion, or a neutral loss mode to obtain an array of all parent ions that lose a common neutral fragment. Further, these scan functions can be changed many times during analysis, so that one can detect and measure butyl esters of acylcarnitines (by the signature ion at m/z 85) and the butyl esters of α-amino acids (by loss of a neutral 102 fragment) in the same sample.

MS/MS thus permits very rapid, sensitive and, with appropriate internal standards, accurate measurement of many different types of metabolites with minimal sample preparation and without prior chromatographic separation. Because many amino acidemias, organic acidemias, and disorders of fatty acid oxidation can be detected in 1 to 2 minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs. Some conditions that can be diagnosed by MS/MS are listed in Table 1, together with the compound(s) on which diagnosis is based.

Amino acid quantitation by MS/MS is more accurate than most methods now in use for newborn screening and would thus provide more specific and sensitive screening for phenylketonuria, maple syrup urine disease, and homocystinuria. Analysis by MS/MS would also permit the screening menu to be expanded to include a number of disorders that are not currently covered (Table 1). Among these are medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and glutaric acidemia type I (GA1), which are relatively common and difficult to detect before the onset of symptoms and whose outcome is substantially improved by early treatment.

Infants with MCAD deficiency seem healthy in early infancy but develop episodes of hypoketotic hypoglycemia during the first years of life; the first episode is fatal in 30% to 50% of patients. Most of these deaths could be prevented if dietary treatment and measures to prevent fasting were begun before the onset of symptoms. Infants with GA1 develop normally until they suddenly develop acute encephalopathy and irreversible striatal damage during the first 2 to 3 years of life. There is increasing evidence that striatal damage can usually be prevented by L-carnitine and vigorous treatment of catabolic episodes if begun before the onset of symptoms.
Organic acidemias

be addressed, because many third-party payers do not cover

their ongoing medical and nutritional care. Reimbursement
tist, and genetic counselors will be needed to deal with

dified each year by 50% to 100%, and more physicians, nutri-

tionists, and report technical and clinical results.

other screening components, however, e.g., patient retrieval,

cost on the order of $10 per sample. It is important to note

that MS/MS cannot replace current

programs, provided that sufficient funding is

tailed into newborn screening programs, and children that are not detected as

were diagnosed later in life. Thus, as with all newborn

methods, screening should be accompanied by fol-

sary personnel, medications, and medical foods. Indeed, the

made available to cover the costs of the additional and neces-

small. As was the case with all current screening methods, the

beit mostly with patients outside of the immediate newborn

false positives is collected. These considerations argue for pilot

tion of diagnosis, treatment, etc., would vary.

Several issues must be considered before MS/MS is added to

screening for a particular disorder.

and early diagnosis can avoid trauma and expense to the family

families if thoughtfully integrated into new-

The issue of informed consent for MS/MS screening is com-

plicated, in part because uniformly effective therapies have not

veloped for all the conditions the methodology can detect and because it may detect previously unrecognized me-

and allow options for family planning to be considered before

other affected siblings are born.

In summary, MS/MS can provide substantial benefits to pa-

tients and their families if thoughtfully integrated into new-

screening programs, provided that sufficient funding is

made available to cover the costs of the additional and neces-

sary personnel, medications, and medical foods. Indeed, the

expense and complexity of the instrumentation and the need

for trained metabolic physicians to care for the additional pa-

popularized maternal 3-methylcrotonyl-CoA carboxylase
deficiency by acylcarnitine screening of newborn blood spots.7

However, the computer parameters of the MS/MS can be set to

ignore certain molecular ions if a decision is made not to screen

for a particular disorder.

References

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diagnosis of maple syrup urine disease in blood spots from newborns by tandem

It is important to note that MS/MS cannot replace current

programs to screen for biotinidase deficiency, hypothyroid-

ism, hemoglobinopathies, virilizing adrenal hyperplasia, and
galactosemia; these conditions cannot be identified by MS/MS
at this time and must be detected by other means.

Several issues must be considered before MS/MS is added to

ongoing newborn screening programs. The instrument itself,

including the computer and autosampler, is expensive, access
to alternate instruments is imperative in the event of break-
down, and laboratory personnel must be trained extensively to
operate and maintain it. Nonetheless, if the cost of instrumenta-
tion is amortized over several years, MS/MS probably can be

added to existing newborn screening systems for an incremental
cost on the order of $10 per sample. It is important to note
that the cost of screening itself would be the same regardless of
the number of tests added to the screening menu. Costs for
other screening components, however, e.g., patient retrieval,
veriﬁcation of diagnosis, treatment, etc., would vary.

The inclusion of additional disorders in the newborn
screening menu could increase the number of patients identi-
ﬁed each year by 50% to 100%, and more physicians, nutrition-
stions, and genetic counselors will be needed to deal with
their ongoing medical and nutritional care. Reimbursement
for the medical foods needed to treat these disorders must also
be addressed, because many third-party payers do not cover

medical foods, and state laws and regulations regarding reim-
bursement vary.

It has been argued that MS/MS analysis should not be used
in newborn screening until more is known about its sensitivity
(false negatives) and speciﬁcity (false positives) for each of the
diagnosable disorders. Extensive experience with MS/MS, al-
beit mostly with patients outside of the immediate newborn
period, has shown that the number of false positives is very
small. As was the case with all current screening methods, the
number of false negatives will only be learned after newborn
screening is implemented, and children that are not detected as
newborns are diagnosed later in life. Thus, as with all newborn
screening methods, screening should be accompanied by fol-

The issue of informed consent for MS/MS screening is com-
plicated, in part because uniformly effective therapies have not
been developed for all the conditions the methodology can
detect and because it may detect previously unrecognized me-

<table>
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<th>Disorder</th>
<th>Diagnostic metabolite</th>
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<tr>
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<td>Phenylnalnine &amp; tyrosine</td>
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<td>Maple syrup urine disease</td>
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<td>VLCAD deficiency</td>
<td>C₁₄,₁₆,₁₈ acyl- and 3-hydroxy acylcarnitines</td>
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<td>Glutaric acidemia type II</td>
<td>Glutarylcarnitine</td>
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<tr>
<td>CPT-II deficiency</td>
<td>C₁₄,₁₆,₁₈,₂₀ acylcarnitines</td>
</tr>
</tbody>
</table>

Table 1
Some disorders detectable by tandem mass spectrometry

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