ABSTRACT

OBJECTIVE. In response to a California legislative mandate, a pilot tandem mass spectrometry (MS/MS) screening program was undertaken by the Genetic Disease Branch of the California Department of Health Services between January 2002 and June 2003. This article outlines the Genetic Disease Branch approach to implementing the MS/MS pilot program and the program evaluation strategies used.

METHODS. Through the use of multiple data collection methods, we were able to describe hospital participation patterns, screening test uptake, screening test performance, follow-up services utilization, and provider and family satisfaction with the educational materials and follow-up services provided.

RESULTS. During the 18-month pilot program, just more than one half of California’s 755,698 newborns were offered MS/MS screening; among this group, 90% of parents chose to have their newborns screened. Fifty-one newborns were identified with MS/MS-detectable disorders, among 461 patients referred for follow-up testing (0.13% of the screened population). One disorder was diagnosed successfully for every 6939 newborns screened and for every 9 infants referred (excluding phenylketonuria). The overall California population prevalence of MS/MS-detectable disorders was 1 case per 6500 infants (excluding phenylketonuria). The positive predictive value for medium-chain acyl-CoA dehydrogenase deficiency was 86.7%, whereas the positive predictive value for short-chain acyl-CoA dehydrogenase deficiency was 21.6%. For a sample from Hawaii, 1 isovaleric aciduria case was detected among 6132 newborns.

CONCLUSIONS. Evaluation of the California MS/MS screening pilot program demonstrated that this technology was effective in identifying additional metabolic disorders. The positive predictive value of screening was particularly good for medium-chain acyl-CoA dehydrogenase deficiency. Overall, patient referral rates were very acceptable. The utility of the program was also demonstrated by positive reviews from patients and providers.
An increasing number of reports on the use of a new technology; tandem mass spectrometry (MS/MS), as a technique for newborn screening1–3 have stimulated national interest in adding MS/MS to state newborn screening programs.4–6 This technology, which uses a tiny amount of blood, can rapidly identify and measure the concentrations of a large number of amino acids, fatty acids, and organic acids. This method has the potential to detect >30 rare genetic metabolic disorders. Many state newborn screening programs have already implemented or have begun to develop newborn screening methods using MS/MS technology, under increasing pressure from parent and consumer advocacy groups,7 and the need for expansion of newborn screening programs was the focus of several reports in the popular press in 2004.8,9

In response to a California legislative mandate, a MS/MS pilot project was undertaken by the Genetic Disease Branch (GDB) of the California Department of Health Services between January 2002 and June 2003. Because the pilot project was planned as a research study, written informed consent was required for participation. Participation was voluntary, and no additional fee was charged for the supplemental testing. The purpose of the pilot project was to gain practical experience in using the new technology and to evaluate the overall effectiveness of the new test. The experience would help determine what disorders to screen for and how best to incorporate the new technology into the existing mandatory newborn screening program.

The evaluation component examined several aspects of program effectiveness, including participation and screening uptake, specimen throughput, screening test performance, follow-up services utilization, and provider and family satisfaction, by using novel primary data collection strategies, focus groups, surveys, and one-on-one interviews. This article outlines the program evaluation strategies used and the results obtained in California’s MS/MS pilot program.

METHODS

Program Initiation and Informed Consent Procedure

MS/MS screening became available as a research project in California beginning January 7, 2002. Participation in the MS/MS screening pilot program depended on the cooperation of maternity hospitals throughout the state. If a hospital did not participate in the pilot study, then the families of the infants born at that institution did not have the opportunity to be offered MS/MS screening.

Before program start-up, the GDB initiated a large-scale education campaign aimed at maternity hospitals and prenatal and pediatric care providers. Hospital staff members at 299 maternity hospitals throughout the state were asked to distribute an educational booklet that contained information about the California newborn screening program, as well as a section about the MS/MS screening pilot study. The booklet, titled Important Information for Parents, contained the MS/MS informed consent document that parents could sign if they wanted their infants to undergo MS/MS screening. Depending on the parent’s decision, “yes” and “no” stickers contained in the booklet needed to be transferred to the filter paper blood collection card. A detailed tracking system was established to monitor MS/MS test uptake at each maternity hospital and across the state as a whole. Human subject clearance was obtained from the California Committee for the Protection of Human Subjects, and the GDB received a clearance from the Health Resources and Services Administration that allowed cooperating institutions to accept the State of California institutional review board approval. Research staff members set up a system to keep track of which hospitals chose to participate in the pilot study.

MS/MS Laboratory Methods

Four MS/MS systems, ie, 2 API 3000 systems (Applied Biosystems, Foster City, CA) and 2 MSQ 2000 systems (PerkinElmer Life Sciences, Shelton, CT), operated in the positive ion mode (source voltage: 5500 V), were used in this study for the analysis of amino acids and acylcarnitines to detect metabolic disorders classified as amino acid disorders, organic acid disorders, or fatty acid disorders. The systems were matched for their analytical performance before newborn specimens were tested. The mass calibration of each MS/MS instrument was acquired with polypropylene glycol standards (supplied by Applied Biosystems) (m/z 59, 175, 616, 906, 1254, 1545, 2010, 2242). The study tested newborn specimens with the NeoGram amino acids and acylcarnitines derivatized MS/MS reagent kit (PerkinElmer).

The laboratory analysis was performed with 3.2-mm filter paper disks punched from dried blood spot specimens and extracted with a methanol/water (75:25) solution containing stable isotope-labeled internal standards. The samples were derivatized with butanolic HCl, dried at 60°C, and reconstituted with acetonitrile/water (75:25) solution containing acetic acid. A 10-μL aliquot of the sample extract was injected into each of the MS/MS systems, coupled directly to a Gilson 215 autosampler (Gilson, Middleton, WI) and a Shimadzu liquid chromatography pump (Shimadzu Scientific Instruments, Columbia, MD) (pressure: 40 psi; solvent flow rate: 100 μL/min) for the MS/MS API 3000 instrument and a Gilson 215 autosampler and a PerkinElmer liquid chromatography pump (pressure: 40 psi; solvent flow rate: 75 μL/min) for the MSQ 2000 instrument. The MS/MS instruments were optimized by the built-in algorithm to monitor energy transitions from 42 analytes in the neutral loss (m/z 102) mode for amino acids, precursor ion (m/z 85.1) mode for acylcarnitines, and multiple reaction-monitoring mode for certain other
amino acids and acylcarnitines. The analyte concentrations were measured by comparing the instrument responses for each amino acid and acylcarnitine with the responses for the corresponding stable isotope-labeled internal standards.

Establishment of Analyte Cutoff Values
Although we examined all of the available literature from other states and private laboratories that were experienced in MS/MS screening, during analysis of anonymous specimens we found that we could not use other programs’ cutoff values in California, possibly because of different laboratory methods and population-based differences in analyte distributions. We therefore used a multistep process to establish cutoff values.

The first exercise was to evaluate 10 000 anonymous prepilot specimens on the basis of statistical analysis of the analyte value distributions. The analysis focused on the tails of the distributions and percentiles, projected positive rates and workload, analyte-by-analyte considerations (such as a higher cutoff value for tyrosine because of a high false-positive rate), and whether a given analyte was a primary or secondary marker for clinical disease. Using this method, we set cutoff values for 24 acylcarnitines (including upper and lower cutoff values for free carnitine and acetylcarnitine) and 14 amino acids. An additional 6 acylcarnitines were reported routinely, but we did not think we had appropriate data for setting cutoff values (or metabolic advisors thought that some analytes were not clinically significant). After these proposed cutoff values were reviewed with our metabolic specialist advisory committee, they were implemented at program initiation in January 2002.

We reevaluated cutoff values after 3 months, when we had tested ~40 000 specimens. These analyses, including examination of realized positive rates and another meeting with the metabolic advisory committee, led to additional changes. Cutoff values were eliminated for valine, glutamate, aspartate, and hydroxyoctanoylcarnitine.

Cutoff values were evaluated a third time with the same factors, based on ~60 000 specimens, in May 2002. These changes included raising some cutoff values (acetylcarnitine, propionylcarnitine, isovalerylcaritnine, and hydroxyoctanoylcarnitine) and lowering some (myristoylcarnitine, hydroxydecanooylcaritnine, and hydroxyoctanoylcaritnine). Furthermore, cutoff values were added for some analytes that had none previously because of inadequate data (malonylcarnitine, lauroylcaritnine, and 3-OH-tetradecanoylcarnitine).

Another approach to testing the appropriateness of our analyte cutoff values was to conduct MS/MS testing of known cases. We performed MS/MS analysis of samples from frozen dried blood spots for a total of 45 known cases before the pilot program began.

Initially, a valine cutoff value and a leucine/isoleucine cutoff value were used for maple syrup urine disease (MSUD). However, the valine cutoff value was eliminated by metabolic specialist request because it was generating large numbers of false-positive results. Of the 5 known cases of MSUD (from screened and stored specimens), all would have been detected with the valine cutoff value; however, because it was dropped, we found that the leucine/isoleucine cutoff value of 350 μmol/L would detect only 3 of 5 cases. We then implemented a new method that included a leucine cutoff value of 300 μmol/L and a leucine/alanine ratio of >1.75. With this method, all 5 cases were detected without an unacceptably high false-positive rate.

For case ascertainment that was as complete as possible, to establish appropriate cutoff values and to establish the incidence rates of MS/MS-detectable disorders in the population, we asked the metabolic centers to report to us all new confirmed cases identified during the 18-month pilot program period. After we obtained signed informed consent, we performed MS/MS analysis of samples from the diagnosed cases, to validate our cutoff values. To obtain the most-accurate prevalence estimates, we also requested that California coroners report to us any unexplained deaths that occurred during the pilot testing period, so that MS/MS analysis could be conducted.

MS/MS Testing Process
All MS/MS specimens were tested at the Genetic Disease Laboratory (GDL), after the mandatory newborn testing was completed at 1 of 8 newborn screening laboratories throughout the state. Specimens were held at the contract laboratory until the mandatory newborn screening assay was complete and then were mailed to GDL for MS/MS testing.

Test results were transferred electronically to the MS/MS database once each day. All initially positive test results were reviewed by a clinical chemist at GDB, who decided whether the case should be referred for follow-up testing. Clinically insignificant results were reclassified to reduce the rate of false-positive referrals. In some cases, mild elevations, indications of hyperalimentation, or elevated analyte levels not associated with clinical disease were not referred. All potentially clinically significant results were referred to metabolic specialists at 1 of 14 metabolic centers across the state. Qualified specialists at the centers were able to confirm or to rule out the diagnosis of a condition and, when indicated, to initiate appropriate prevention measures or treatments to minimize the impact of the disorder.

MS/MS Case Referral
Two coordinators were hired to track the newborns who were referred for follow-up evaluation. After review and release of the case by the clinical chemist, a daily, computer-generated report identifying new case referrals...
was released to the coordinators. The coordinators were responsible for initiating the follow-up process by sending a test result mailer to the pediatric provider (sent only for positive cases) and facilitating communications between the patient, pediatric provider, and metabolic center. Coordinators were responsible for monitoring and documenting the details of their patient follow-up correspondence in the project database. They tracked the patients until a definitive disorder was either determined or ruled out, and they closed the case in the database as “resolved-normal,” “resolved-abnormal,” or other final case status categories (e.g., “no resolution: patient declined service” or “no resolution: patient lost-to-follow-up”).

Screening of Newborns From Hawaii
A 3-state collaboration involving the Hawaii newborn screening program, the Oregon Public Health Laboratory, and the California GDB was formed to screen newborns from Hawaii. Beginning March 2002 and continuing through middle June 2003, staff members at the Hawaii newborn screening program offered MS/MS supplemental screening to newborns born at Kapiolani Medical Center for Women and Children, with an informed consent process similar to the California protocol. After routine newborn screening in Oregon, MS/MS specimens were sent to the California GDL. Each week, California staff members sent a summary of test results to the Hawaii newborn screening coordinator. Because Hawaii did not have a metabolic specialist during the pilot testing period, a California-based metabolic specialist was contracted to provide follow-up consultation and coordination for referred patients and to work directly with families when necessary. Health Resources and Services Administration grant funds were used to cover the logistic costs of specimen identification and data transfer for the group of women who opted to have MS/MS screening, specimen transfer from Oregon to California, and the metabolic specialist services.

Metabolic Center Reporting Requirement
Metabolic centers were required to send to the GDB a document that indicated the final diagnostic status of each infant referred for follow-up testing. In addition, metabolic centers were required to complete a MS/MS patient contact form that documented the type and quantity of the services provided and the tests ordered for each MS/MS screen-positive newborn referred to the clinic during the pilot screening period. As part of the patient contact form, metabolic specialists documented the overall health status of the infant and noted any expressed parental concerns at the time of each patient contact. Program evaluation staff members collected information about the type of follow-up tests ordered and the test results, and the data were entered into the MS/MS screening database.

Overview of Program Evaluation Strategies
Research staff members used several strategies to assess the effectiveness of MS/MS screening from the patients’ and providers’ perspectives. Investigations included a survey of California prenatal care providers to assess their knowledge of and attitudes about MS/MS screening; focus groups with pregnant women to evaluate the clarity, adequacy, and effectiveness of the informed consent process; interviews with parents of newborns who were referred for follow-up testing; and a survey of pediatric providers with patients who required follow-up testing. A summary of the methods and findings of the pediatric care provider survey are presented here, and details of the other investigations will be reported elsewhere.

Pediatric Care Provider Survey
A 2-page survey instrument was mailed to 325 pediatricians who had an infant referred for follow-up testing during the pilot program period. Pediatricians were queried regarding their level of knowledge about MS/MS screening and their satisfaction with the state follow-up coordinators and metabolic centers, how prepared they felt initially to answer patient questions about the positive MS/MS screening test result, and which sources were most useful for obtaining information about MS/MS-detectable disorders.

RESULTS
Maternity Hospital Participation
MS/MS screening was available to newborns from January 7, 2002, through June 13, 2003. During this period, there were 755 698 births in California.

Hospital participation throughout the 18-month pilot period was incomplete and varied widely. By tracking the yes and no stickers on the filter paper blood collection card, we were able to determine that no hospital offered MS/MS screening to 100% of their newborns and 20% (n = 63) offered it to none of their newborns. Figure 1 shows the percentage of hospitals according to the level of participation. The largest group of hospitals (n = 92; 31% of all participating hospitals) offered MS/MS screening to 50% to 74% of all newborns delivered at that institution; 68 hospitals (23%) offered MS/MS screening to ≥75% of all newborns.

MS/MS Screening Test Uptake
Of the 755 698 births in California during the pilot program period, 52% of all newborns were offered MS/MS screening; 47% of all families opted to have the test and 5% of families declined screening. Among all families offered MS/MS screening, 90% opted to have the test. The 90% overall screening uptake rates were similar across all race/ethnic categories. In addition, the race/ethnic profile of the MS/MS-screened population was
similar to the race/ethnic distribution of the population of California newborns.

**Performance of MS/MS Testing Equipment and Specimen Throughput**

The performance of the MS/MS testing method was evaluated with National Committee for Clinical Laboratory Standards protocol EP10A. The average recoveries of amino acids and acylcarnitines were 94% and 96%, respectively. The average intraassay and interassay precisions were 11.7% and 12.0% for amino acids and 18.8% and 18.0% for acylcarnitines, respectively. The method showed high sample throughput, wide analytical range, and linear relationship ($r^2 > 0.90$). The carryover from sample to sample was statistically insignificant ($P > .01$) and thus had no effect on individual analytic results. A separate study to compare the efficacy of derivatized versus nonderivatized methods demonstrated that, although there are some differences, either method for the analysis of amino acids and acylcarnitines is suitable for screening clinical specimens for the detection of metabolic disorders.

**MS/MS Screening Test Performance**

During the first 5 months of screening, our initial MS/MS positive rate (initially flagged specimens) was 0.49%. After the final cutoff value changes (described above) were implemented, the rate decreased to 0.07%. In the entire 18-month pilot test period, 701 cases (0.20% of screened cases) had an initially positive flag; when positive results were eliminated after clinical review, however, only 461 cases were referred for follow-up services (0.13% of screened cases). This translates to 1 newborn referred for every 768 newborns screened.

Figure 2 presents a schematic diagram of the patient testing and follow-up process. A total of 51 newborns with confirmed disorders were reported among the 461 referred patients. On the basis of our actual screening experience, 1 case was diagnosed for every 6939 newborns screened and 1 disorder was diagnosed for every 9 infants referred. With the 3 additional missed cases (2 of which would have been detected with our revised cutoff values), the California population prevalence of MS/MS-detectable disorders was 1 case per 6500 infants (excluding phenylketonuria).

Table 1 presents an overview of MS/MS-detectable disorders that were diagnosed during the California pilot study and the respective population prevalence rate for each disorder. The disorders with the highest prevalence rates were short-chain acyl-CoA dehydrogenase deficiency (SCADD), medium-chain acyl-CoA dehydrogenase deficiency (MCADD), and methylmalonic acidemia/propionic acidemia (MMA/PA). Details of the 3 missed cases are described below.

The clinical chemist who reviewed all of the cases with any initially positive flags was able to record in the MS/MS database the disorder he suspected at the time the referral was made. With assignment of these categories (eg, suspected MCADD), we were able to determine the screening test performance and positive predictive values according to disorder.

Table 2 presents the screening test performance for the various disorders detected during the pilot screening test. Positive predictive values were particularly good for MCADD, with 13 cases being detected among 15 referrals for suspected MCADD. Other disorders had lower positive predictive values, ie, 21.6% for SCADD, 20.0% for glutaric acidemia (GA)-I and 3-methylcrotonyl-CoA carboxylase deficiency (3MCC), and 12.0% for MMA/PA.

**Documentation of Unscreened and Missed Cases and Input From California Coroners**

A total of 14 cases were reported to us from the unscreened population during the pilot study (MCADD: 2 cases; MSUD: 3 cases; MMA/PA: 4 cases; GA-I: 2 cases; isovaleric aciduria: 1 case; long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency [LCHADD]: 1 case; carnitine palmityl transferase deficiency type II/carnitine-acylcarnitine translocase deficiency: 1 case). If the prevalence of disorders was the same as in the screened population (1 case per 6500 infants), then there were 61 affected newborns in the unscreened cohort. This means that
$77\%$ of affected newborns will not be identified in the absence of screening. At the time of this writing, no additional unscreened cases have been reported to us. Three cases in the screened population were not detected. One case of LCHADD would have been detected with the revised cutoff value, which was already in place before the reporting of this case. One case of MSUD would have been detected with our revised ratio and leucine cutoff value. One case of very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) was diagnosed prenatally and was being treated; therefore, the deficiency was not detectable during the first month by our program or by 2 other established laboratories to which we sent the specimen.

Four additional disorders, which are not usually on the list of metabolic disorders detected with MS/MS screening, were detected during the pilot study, ie, biotin metabolic defect (elevated C5OH level), mitochondrial energy metabolism deficiency (elevated glycine level), methionine adenosine transferase deficiency (not being treated; elevated methionine level), and hypermethioninemia (typically benign; elevated methionine level).

Our effort working with the California coroners revealed no new MS/MS-detectable cases. California coroners identified 16 newborns with an unknown cause of death.

### Table 1: Overview of Disorders Identified Through the California MS/MS Pilot Screening Program ($n = 353,894$ Newborns Screened)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. of Cases</th>
<th>Prevalence (Screened Population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCADD</td>
<td>13</td>
<td>1 case per 27,000</td>
</tr>
<tr>
<td>SCADD</td>
<td>18</td>
<td>1 case per 20,000</td>
</tr>
<tr>
<td>MMA/PA</td>
<td>11</td>
<td>1 case per 32,000</td>
</tr>
<tr>
<td>LCHADD</td>
<td>1</td>
<td>1 case per 354,000</td>
</tr>
<tr>
<td>GA-I</td>
<td>2</td>
<td>1 case per 177,000</td>
</tr>
<tr>
<td>GA-II</td>
<td>1</td>
<td>1 case per 118,000</td>
</tr>
<tr>
<td>3MCC</td>
<td>1</td>
<td>1 case per 354,000</td>
</tr>
<tr>
<td>Argininemia</td>
<td>1</td>
<td>1 case per 177,000</td>
</tr>
<tr>
<td>VLCADD</td>
<td>1</td>
<td>1 case per 177,000</td>
</tr>
<tr>
<td>MSUD</td>
<td>1</td>
<td>1 case per 6,500</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>1 case per 6,500</td>
</tr>
</tbody>
</table>

*Not detected with the January cutoff value but detected with the revised cutoff value.

### Table 2: Screening Test Performance for Disorders Detected in the California MS/MS Screening Program (January 7, 2002, to June 13, 2003)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. of Cases</th>
<th>No. of Referrals</th>
<th>Positive Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCADD</td>
<td>13</td>
<td>15</td>
<td>86.7</td>
</tr>
<tr>
<td>SCADD</td>
<td>18</td>
<td>90</td>
<td>21.6</td>
</tr>
<tr>
<td>MMA/PA</td>
<td>11</td>
<td>51</td>
<td>12.0</td>
</tr>
<tr>
<td>3MCC</td>
<td>3</td>
<td>25</td>
<td>20.0</td>
</tr>
<tr>
<td>GA-I</td>
<td>1</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>GA-II</td>
<td>2</td>
<td>2</td>
<td>100.0</td>
</tr>
<tr>
<td>MSUD</td>
<td>1</td>
<td>40</td>
<td>2.5</td>
</tr>
<tr>
<td>VLCADD</td>
<td>1</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td>Argininemia</td>
<td>1</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td>Other referrals</td>
<td>0</td>
<td>201</td>
<td>0.0</td>
</tr>
</tbody>
</table>

$S266$
death during the pilot testing time frame. MS/MS analysis was conducted with the dried blood spots for these infants, and all test results were negative.

**Summary of Multistate Collaboration With Hawaii**
As part of the collaboration, 6132 newborns from Hawaii were screened during California’s MS/MS pilot screening program. Six cases had initially positive analyte flags; after review by the clinical chemist, 2 cases were referred to the Hawaii newborn screening coordinator for follow-up evaluation. One case of isovaleric aciduria was diagnosed eventually.

**Overview of Findings on Utilization of Follow-up Services**
Among the 461 referrals made for follow-up services, we received (to date) 721 patient contact forms, representing 443 patients (a small number of these indicated cases lost to follow-up monitoring). A preliminary analysis revealed that 72% of the referred patients had 1 contact with the metabolic center and 6% had ≥4 contacts. In contrast to the entire group, 7% of newborns referred for suspected MCADD had 1 contact with the metabolic center and 46% had ≥4 contacts.

The utilization data showed that most of the referred patients were never actually examined at the metabolic follow-up center (only 35% of all first patient contacts were actually made at the metabolic clinic), which indicates that the centers were able to work with the newborns’ primary care physicians to order and to evaluate initial diagnostic tests. The data also showed that, although most disorders were diagnosed among symptomatic newborns with abnormal diagnostic test results, a significant number of disorders were eventually confirmed among newborns who were asymptomatic at the time of referral. A more-complete presentation of the findings of this investigation is being prepared for publication.

**Overview of Findings From the Pediatric Care Provider Survey**
Surveys were mailed to 325 pediatricians who had an infant referred for follow-up testing; 124 (38%) of the surveys were returned. The physicians rated highly the ability of the nurse coordinators to inform them about the follow-up process, to answer questions about the screening program, and to help them coordinate the steps to ensure that the infant received follow-up services.

When asked who first notified the family about the positive MS/MS screening test results, 45% of the pediatricians said that they first spoke with the family, 31% said that the MS/MS coordinator first spoke with the family, 13% said that their staff members spoke with the family, and 11% said the metabolic center made the first program contact. Only 46% of pediatricians said they felt very well or moderately well prepared to answer the family’s questions about the abnormal test result during the first conversation with parents; 42% said they felt very or moderately unprepared at that time. After working with the follow-up coordinators and the metabolic centers, the percentage of pediatricians who felt very well or moderately well informed increased to 52%; however, 24% of the providers still felt that they were not very well informed about the disorder for which the patient was referred.

Only 67% of the pediatricians who were identified as the primary care provider at the time of birth stated that they were still the primary care provider managing the care of the infant at the time of the survey. Among respondents, 13% said that they no longer manage the care of the infant and, surprisingly, 20% said that they never managed the care of the infant. The survey results demonstrated that the pediatric providers who were still providing care to the newborns who were referred for MS/MS follow-up services were mostly satisfied with the follow-up services provided; however, a surprising number said that they never or no longer managed the newborn’s care. A more-detailed description of the survey findings is being prepared for publication.

**DISCUSSION**

**Program Achievements**

**Low False-Positive Rate**

One concern was that, with so many conditions, families would be told that their newborn was affected only to find, after diagnostic testing, that the results were normal variations. As a result of the information collected in the pilot project, the GDB was able to reduce this problem of false-positive referrals from an initial figure of 0.49% to a final figure of 0.07% during the last year of the trial program. The final false-positive rate is a very acceptable rate for a population-based screening program. The rates for the disorders screened for with MS/MS may vary, but the overall rate is the important outcome.

**Disorder Prevalence Rate**

The MS/MS technology detects some disorders better than others. The overall MS/MS-detectable disorder prevalence rate was 1 case per 6500 newborns screened in the MS/MS pilot project (this includes the 3 missed cases). Projections of the disorder prevalence rate expected when phenylketonuria is included in MS/MS screening suggest 1 MS/MS-detectable disorder for every 4463 infants screened. This assumes a total of 121 disorders (83 nonphenylketonuria disorders plus 19 classic and 19 variant phenylketonuria cases) among 540 000 newborns screened.

**Program Acceptable to Pregnant Women**

As described in another manuscript, we found that there was considerable support for the screening pilot
study, on the basis of our focus groups with 31 pregnant women and 200 parent interviews. Most women appreciated the benefits that screening could offer, and >90% of women said they would participate again if they had the choice to make again. A large proportion of women said that they expect their prenatal provider to inform and to advise them about newborn screening.

Program Acceptable to Pediatric Providers
Pediatric providers responded positively to the MS/MS screening pilot program and, despite the fact that many providers initially did not know much about the disorders detectable through MS/MS screening, the MS/MS follow-up coordinators and the metabolic center staff members were able to coordinate patients’ follow-up services effectively, to educate pediatricians about the disorders suspected, and to help pediatricians answer questions raised by the families. These findings emphasize the need to include metabolic centers or experts in the follow-up evaluation of positive screening results.

Program Challenges

Informed Consent Issues
Many hospitals refused to participate because of the requirement for informed consent. As a result, screening was available to only approximately one half of California newborns. This would not be an issue with mandatory screening, but the experience should be noted for those advocating informed consent in population-based newborn screening.

MS/MS Specimen Testing Issues
California decided to use and to report the entire spectrum of analytes identifiable with MS/MS methods, because we thought this would allow for collection of more data that could be used to make an informed decision regarding which analytes to include in the permanent program. It is also consistent with the American College of Medical Genetics report on uniform screening. It was thought that there were ethical concerns involved with not reporting other elevated values and that use of the multiple reaction-monitoring technique would not lead to a robust evaluation of the technology. Furthermore, metabolic specialists throughout the state made a very strong case in requesting that we include all analytes and follow up on all positive results to collect data on lesser-known metabolic disorders. Result mailers included all analyte names, values, positive flags, and reference ranges.

MS/MS Cutoff Challenges
As described in the Methods section, cutoff determination must be considered an ongoing process, with periodic evaluation and reevaluation based both on data collected and on input from metabolic specialists. Positive rates, analyte values of missed cases, and workload considerations should all be used in these evaluation schemes.

Missed Expected Cases in the Unscreened Population
The significance of the missing expected cases in the unscreened population is not trivial. As noted earlier, only 14 (23%) of 61 expected cases in the unscreened population were reported to us. Future state pilot programs will need to weigh the benefits of gaining experience to improve screening test performance before incorporating a new technology into mandatory newborn screening versus the risk of missing cases during a pilot research phase of investigation.

Most of the discrepancy with respect to the 47 expected unreported (unscreened) cases is explained by 2 factors. The first is MCADD (13 vs 2 cases); because many cases of MCADD do not have significant symptoms during the first year, it seems that these cases in the unscreened population have not yet been diagnosed on the basis of clinical symptoms. The second is SCADD (18 vs 0 cases); SCADD detection is difficult because many of the cases are mild or without symptoms and thus may never be reported if newborn screening is not performed. It is not known whether any of the 18 cases we detected will have serious symptoms. Almost all affected infants underwent mutation analysis. Most had 1 or 2 copies of the polymorphism, and only 1 had the disease-causing mutation.

Utilization of Screening
The experience with hospitals offering voluntary supplemental newborn screening indicates the superiority of a state-mandated screening system over privately provided screening. Although 99.8% of newborns received the state-required screening panel, supplemental non-mandated screening was seriously underutilized, which resulted in many newborns not being screened. This would be especially true if there is an additional collection of blood from the newborn and an additional charge for the supplemental screening that affects the uninsured disproportionately.

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