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The mission of the American Society for Clinical Laboratory Science is to promote the profession of clinical laboratory science and provide beneficial services to those who practice it. To enable its members to provide quality services for all consumers, the society is committed to the continuous quest for excellence in all its activities.

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The Hard Work of Advocacy

KATHY HANSEN, DON LAVANTY

Following the 2002 Legislative Symposium, the ASCLS Government Affairs Committee (GAC) has continued to work on the advocacy issues that we raised as they continue their respective journeys through the Congress.

There were five ‘leave-behind’ issue papers addressing legislative and regulatory issues of interest to the laboratory. We have had a great success on the Bioterrorism/Personnel Shortage legislation and some success on the Medicare Reimbursement. We also continue to work on the other three issues: specimen collection fee, Medicare simplification, and criteria for waived test classification.

Bioterrorism and the personnel shortage
As unfortunate as the events related to September 11th were, the memory of the threat of anthrax on Capitol Hill provided an effective vehicle to use to advocate for support to alleviate the laboratory personnel shortage. Legislative Symposium attendees generally found Senate and House offices to be sympathetic to concerns about allied health shortages and infrastructure. We were making our visits at a time when the conference committee was beginning to work on a compromise version of House and Senate-passed bills.

Ultimately, the conference committee of the House and Senate agreed upon the items found in the House-passed version of the bioterrorism bill that provide for financial support to train additional laboratory professionals. This support is in the form of loan forgiveness, grants to programs, scholarships, and other incentives. That compromise version was passed and signed into law by President Bush. A great success for those who worked so hard at Legislative Day and one we can be proud of!

The work has not ended, however. ASCLS is currently lobbying House members, particularly members of the House Subcommittee on Labor, Health and Human Services, and Education to include in the 2003 budget the appropriations for clinical laboratories in the Clinical Laboratory Coalition lobbied hard against organizations in the Clinical Laboratory Coalition.

Reimbursement for specimen collection
In 1984, the Medicare program established a rate of $3.00 for outpatient blood collection (includes hospital outpatients, clinic patients, and nursing homes). That fee has stayed the same for 17 years. Obviously, during that time salaries have increased and supplies have become more costly in order to meet current safety requirements. There was a bill introduced in the House (H3448), which would raise this fee to $5.25. There is not yet a companion bill in the Senate. If passed, this increase will result in $600 million in additional national laboratory revenue over five years. Current status of this bill is not optimistic.

Allow inflationary increase for clinical laboratory testing reimbursement
In 1997, the Balanced Budget Act imposed a freeze on Medicare fee schedule increases for clinical laboratory services. During that time the Consumer Price Index has increased 1.5% to 2.7% per year, but because of the freeze laboratory reimbursement did not increase. The clinical laboratory is the only segment of healthcare that has actually seen its reimbursement decrease (in real dollars) since 1984. If no Congressional action is taken, the five-year freeze will end at the end of this budget year, so that laboratories can receive an inflationary increase in 2003. However, Secretary Thompson of Health and Human Services suggested renewing current freezes in order to fund a prescription drug benefit.

Since the Legislative Symposium, the House Ways and Means Committee considered a bill that would have continued the freeze on the clinical laboratory fee schedule until competitive bidding for laboratory services could be established (potentially several more years). ASCLS members and members of other professional organizations in the Clinical Laboratory Coalition lobbied hard against this, and again were successful in that the freeze has now been taken out of the proposal. Unfortunately, there is still a provision calling for a competitive bidding demonstration project.

Medicare simplification
The House of Representatives has already passed the Medicare Regulatory and Contracting Reform Act of 2001 (HR 3391), which contains some of the recommendations that were made in the Institute of Medicine’s December 2000 report on laboratory reimbursement. The provisions of most interest are:

- Improving the process for evaluating and pricing new tests, which is of critical importance with the explosion of genetic testing.
- Removing the burdensome requirement for hospital laboratories that serve as reference laboratories for clinics to determine whether Medicare is the patient’s primary insurance. This is ex-
tremely difficult to do when the laboratory is receiving samples and not seeing the patient.

The companion Senate bill, S1545, is being considered by the Senate Finance Committee. We hope that bill will reach the full Senate in the fall.

Waived testing
Under Clinical Laboratory Improvement Amendments (CLIA) regulations, tests are categorized as waived, moderate complexity, or high complexity. Waived tests are unregulated, in the sense that laboratories that perform them are not inspected and need not perform and report proficiency testing. There are no standards for personnel performing waived tests.

The FDA has one set of standards for evaluating waived tests for home use, i.e. over the counter tests, and a more stringent set of criteria for evaluating waived tests intended for diagnostic use, such as in a physician office laboratory. However, once a test has been approved as waived it can be used in either setting. There is potential risk to patient safety if an incorrect result is obtained with the less accurate home use test.

The Centers for Medicare and Medicaid Services (CMS), the Food and Drug Administration (FDA), and the Centers for Disease Prevention and Control (CDC) have been charged with developing new criteria for waived test approval. We asked Congress to contact these agencies to advocate for more stringent and safer standards and many of our members received positive responses for support of our position on this issue. (See Winter 2002 Clinical Laboratory Science for more on waived testing.)

The advocacy work that our members do each year at the Legislative Symposium informs Congress about our issues and serves as a base for other forms of advocacy, e.g., letters and emails as well as follow-up calls by our Legislative Consultant, Don Lavanty. ASCLS provides this important service to its members as well as the larger laboratory community. We need to have everyone participate. See the roster for the GAC on the ASCLS Web site for contact information for the person who is GAC liaison to your state and region. Get involved!

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Abstracts must be postmarked by January 15, 2003
Hereditary Deficiencies of Antithrombin III, Protein S, and the Protein C Pathway in Jordanian Thrombosis Patients

SUHAIR S EID

Hereditary thrombophilia is caused by various inherited disorders. Most lead to a familial tendency to recurrent venous, not arterial, thrombosis, usually at a young age, and with spontaneous onset. Most of the genetic defects known today affect the function of natural anticoagulant pathways, in particular, the protein C system.

In this study, 602 (265 female, 337 male) patients with suspected thrombosis, arterial or venous, were referred to King Hussein Medical Center in Amman, Jordan. The prevalence of hereditary deficiencies of antithrombin (AT), protein S (PS), and protein C (PC) were studied over a seven-year period (1993–2000). Activated protein C (APC-R) resistance subjects were studied over four years (1996–2000). The mean age was 30 years in females and 42 years in males. A diagnosis was established in 22.4% (n = 135) of the subjects (20.3% venous, 2.1% arterial). Protein C deficiency was found in 3.8%, protein S deficiency in 2.3% and antithrombin deficiency in 1.4% of our sample group. An APC-R problem was seen in 23.0% (n = 89) of the surveyed population. Out of the APC-R patients, 75.0% had the DNA analysis of a factor V Leiden mutation present. Of the subjects found to have the mutation 87.0% were heterozygous and 13.0% were homozygous. These results confirm that APC-R, as a result of factor V Leiden mutation, is the most prevalent cause of thrombosis, and thrombophilia is related to venous, not arterial, thrombosis.

ABBREVIATIONS: APC-R = activated protein C resistance; ASPCR = allele specific polymerase chain reaction; AT = antithrombin; CVA = cerebral vascular accident; DVT = deep vein thrombosis; FVL = factor V Leiden; N-APC-R = normalized activated protein C ratio; PC = protein C; PCR = polymerase chain reaction; PE = pulmonary embolism; PS = protein S.

INDEX TERMS: coagulation; thrombosis.

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The peer-reviewed Clinical Practice section seeks to publish case studies, reports, and articles that are immediately useful, of practical nature, or demonstrate improvement in the quality of laboratory care. Direct all inquiries to Bernadette Rodak MS CLS(NCA), CLS Clinical Practice Editor, Clinical Laboratory Science Program, Indiana University, 409, 1120 South Avenue, Indianapolis, IN 46202-5113. brodak@iupui.edu

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Thrombophilia is the tendency toward recurrent venous thromboembolism usually occurring in young age. In recent years, important advances have been made in understanding the complexity of laboratory abnormalities and clinical conditions associated with an increased risk of thrombosis.1 The term inherited thrombophilia acknowledges the presence of an inherited factor that by itself predisposes towards thrombosis. However, due to the episodic nature of thrombosis, interaction with components is required before onset of the clinical disorder. The concept of inherited thrombophilia is an operational one. After the discovery of AT, genetic disorders of PC anticoagulant pathway, namely deficiencies in PC or PS, have been demonstrated to be associated with thrombophilia.2 Also the discovery of the inherited activated PC resistance (APC-R), due to a single point mutation in Factor V gene (1691 G-A in exon 10 leading to 506 Arg to Glu) is associated with a significant increase in venous thrombotic risk.3, 5, 7 The presence of APC-R and anticoagulant protein deficiencies does not seem to be a significant risk factor for arterial thrombosis.3, 6, 7

Progress in the molecular basis of thrombosis has enabled a more genetically based definition to be formulated: inherited thrombophilia is a genetically determined tendency toward venous thromboembolism. Dominant abnormalities, combinations, or less severe defects may be clinically apparent from early age of onset, or a frequent recurrence with a family history. Milder traits may be discovered only by further laboratory investigation. All genetic influences and their interaction are not yet understood.8

In previous studies, the prevalence of hereditary thrombophilia among patients with venous thrombosis was found to be greatly dependent on criteria for selection of patients. The aim of this study was to estimate the prevalence of hereditary thrombophilia, and to estimate the prevalence of arterial thrombosis in comparison to venous thrombosis (where it can be shown that thrombophilia is related to venous thrombosis and not arterial thrombosis) in 602 consecutive Jordanian patients referred for evaluation to the hematology department/coagulation section of King Hussein Medical Center.

MATERIALS & METHODS

Patient selection

Of the 602 patients (265 female, 337 male) evaluated in our study, 150 had arterial thrombosis, and 452 had venous thrombosis. These
patients were studied from 1993–2000, for inherited PC, PS, and AT deficiency. Three hundred seventy-nine patients were studied for APC-R from 1996–2000. Of the 379 patients, DNA analysis for FVL mutation was performed for 197 patients.

Patients with malignant disorders were excluded. None of the patients had received heparin or oral anticoagulant for at least three months prior to our investigation. Lupus anticoagulants were excluded. The deficient anticoagulant proteins or APC-R had to be consistently below 2SD of the normal mean on repeated samples. Three months was the period between repeats of the sample analysis.

For this study, thrombophilia was characterized as congenital and not acquired when the deficient protein was constantly below normal levels and when the same deficiency was confirmed in at least one family member. Members of the patients’ families who were found deficient in one protein or had APC-R, were not included in this study.

**Laboratory evaluation**

Blood samples were collected in a 9:1 blood to anticoagulant ratio with 0.13 M trisodium citrate. Platelet poor plasma was prepared by centrifugation for 15 minutes at 2500g. Plasma samples were frozen and stored at –85 °C until assayed.

In all patients, antigenic AT, PC, PS were evaluated using laurel rocket immunoelectrophoresis, immunoturbidometry, and ELISA techniques (Assera-plate, Assera-chrome Liatest, Diagnostica Stago). Functional PC and AT were evaluated using colorometric methods (chromostrate AT, Organon Teknika, Stachrom protein C, Diagnostica Stago) according to manufacturer directions.

All plasmas were diluted in a ratio of 1:5 (1 part plasma: 4 parts FV deficient plasma) and APC-resistance was performed as described previously (APC deficient plasma was purchased from Diagnostica Stago).9

Genomic DNA was prepared from EDTA blood. Determination of the FV Leiden mutation (G to A at position 1691) which causes APC resistance, was performed using two PCR methods. The first PCR method was a restriction enzyme method.4 The second was a multiplex allele-specific PCR method.10

**Controls**

Normal plasma was obtained from 30 normal donors for the various assays. Commercial reference plasmas (Dade-Behring, Diagnostica Stago) were used to confirm the assay results. Reference ranges were established for all anticoagulant proteins and APC-R for healthy controls. The results are as follows: free, total PS, 54% to 140%; PC functional 62% to 138%; antigenic PC 60% to 145%; antigenic AT assay was 82% to 120%; functional AT was 80% to 122%; and for normalized APC ratio: ratio >0.75.

**RESULTS**

602 patients met the criteria of this study, 337 were males, 265 were females. Mean age in females was 30 years (2 to 60). The mean age in males was 42 years (2 to 60). The most predisposing factors in females were pregnancy and oral contraceptives. The sites of thrombosis and the number of patients are shown in Table 1.

Detection of anticoagulant protein deficiency, and/or APC-R was established in 22.4% (n = 135), 122 with venous thrombosis (20.3%), 13(2.1%) with arterial thrombosis, while no abnormality was detected in 79% (n = 476) of the cases. Deficiencies of PS, PC, or AT were found in 7.5%. Thus AT deficiency was found in 1.4% (n = 9): 1.3% were type I, 0.16 were type II; 3.8% (n = 23) had PC deficiency: 3.1% were type I, 0.7% were type II; and 2.3% (n = 14) had PS deficiency, all were type I.

Out of the 379 patients studied from 1996-2000, 23% (n = 89) were found to have APC-R; DNA analysis could only be performed for 75% of the patients. 65 patients proved to be positive for FVL mutation, (87% were heterozygous, 13% were homozygous). In two patients no FVL mutation was detected. Two patients were found to have both PC deficiency type I, and were heterozygous for FVL mutation. Both patients had recurrent DVT, one with PE, the other with a CVA.

After investigation the mean age of patients found to have PC, PS, or AT deficiency was 30.2 years; the mean age of patients found to have APC-R was 32.3 years. Three patients, one displaying a PC

| Table 1. Sites of thrombosis, and type of deficiency in 135 patients found to have hereditary thrombosis |
|-----------------|---|---|---|---|---|
|                | VTH | PC | PS | AT | APC—R | Total |
| DVT             | 13  | 11 | 3  | 65 |       | 92    |
| PE              | 4   | —  | 1  | 9  |       | 14    |
| RA              | 1   | 1  | —  | 8  |       | 10    |
| Others          | 1   | —  | 3  | 2  |       | 6     |
| TOTAL           | 19  | 12 | 7  | 84 |       | 122   |

| ATH             | —   | —  | 2  | 1  | 3     |
| MI              | 4   | 2  | —  | 4  | 10    |
| TOTAL           | 4   | 2  | 2  | 5  | 13    |

ATH = arterial thrombosis; CVA = cerebral vascular accident; DVT = deep vein thrombosis; MI = myocardial infarction; PE = pulmonary embolism; RA = recurrent abortion; VTH = venous thrombosis.

Others: all the rest of the clinical presentations are shown in Table 2.
deficiency, and two with heterozygous FVL mutation, had a first thromboembolic event at ages 60, 58, and 60 respectively.

Table 2 shows the number of patients and the site of thrombosis for each protein deficiency.

DISCUSSION

Congenital AT, PC, PS deficiencies and APC-R (FVL) represent a group of heterogeneous genetic disorders commonly associated with thrombembolic disease. AT is a major protein with inhibitory function on serum proteases in blood coagulation, mainly involving thrombin and factor Xa. PC is a vitamin K-dependent serine protease which together with its cofactor PS, serves as the cofactor of activated protein C (APC), which inactivates factor Va and VIIIa. With the exploration of the anticoagulant protein C system in the 1970s and 1980s, identification of protein C, protein S, and AT deficiencies provided for approximately 10% of cases of familial thrombosis. The real breakthrough in thrombotic diagnosis came in 1993 with the discovery of resistance to activated protein C (APC-R) as a risk factor for thrombosis. In 135 of the original 602 patients (22.4%), a cause of thrombosis was established as an inherited deficiency (20.3% had venous thrombosis; 2.1% had arterial thrombosis). Protein C, PS, AT deficiency and FVL mutation have not been proven to be a cause for arterial thrombosis. These results support the fact that thrombophilia is related to venous thrombosis and not arterial thrombosis.

The prevalence of inherited abnormalities in thrombophilic patients has been investigated in various epidemiological studies. The most important are shown in Table 3. The prevalence of PC and PS congenital deficiencies in patients with a history of thrombosis is slightly higher than the prevalence of AT as noticed in most of the protocols, including the current study. However, studies may vary greatly from one protocol to the other because of different selection criteria and laboratory assays used.

In the current study, 7.5% out of 602 patients studied from 1993–2000 were found to have AT, PC, or PS deficiencies (1.4% had AT deficiency, 3.8% PC, and 2.3% PS). These figures are close to the figures found in different studies done on European and American populations. If we compare this study with a previous study done in Jordan, the percentages were higher than this study. However, there is no defining number in any of the studies.

Most of our patients were from a young age group. These results are very close to the study accomplished by Awidi in Jordan. A high proportion of our patients were younger than 35 years when the first thrombosis occurred, with mean ages of 35.2 years for PS, PC, and AT deficiencies and 32.3 years for APC-R patients. However, three patients, one displaying a PC deficiency and two with heterozygous FV Leiden had a first thromboembolic event at age 60, 58, and 60 respectively. The first one, the PC deficiency patient was diagnosed with portal vein thrombosis. The two FVL mutation patients were diagnosed with DVT. Ten of the females (20%) were suffering from recurrent abortion and were found to have a protein deficiency and FVL mutation, which means that a protein deficiency and the presence of FVL may be a risk factor for recurrent abortion. Therefore all pregnant women with a history of thrombosis should be screened at least for APC-R. Two Budd-Chiari Syndrome patients were found to have a FVL mutation present.

The two most common defects found to be a risk factor for thrombosis in this study were APC-R and FVL mutation. Of the 379 patient studies from 1996–2000, 23% were found to have APC-R. It was then found that 75% of the 89 patients with APC-R that were screened for FVL mutation were found to be positive (87% were heterozygous and 13% homozygous). These results are close to the figures found by the studies in Table 3.

This study shows clearly that hereditary causes of thrombophilia are common in young Jordanian patients with a positive family history of venous thrombosis and not arterial thrombosis. APC-R, FVL accounts for most of these.

ACKNOWLEDGEMENT

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REFERENCES


Table 3. Prevalence of hereditary thrombophilia in selected studies

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Cultural Competency in the Laboratory

CHERYL R CASKEY

Cultural competency incorporates an awareness of different cultures, and sensitivity to the differences of cultures which results in appropriate behaviors with other cultures. White non-Hispanics currently comprise almost 73.6% of the total United States population, but this percentage will decrease to less than 53% by 2050. Laboratory practitioners must become culturally competent to deal effectively with the changing demographics. Cultural diversity will impact the laboratory workforce and the patients it serves. Laboratory professionals must become skilled in communication with all patient populations served, be aware of personal biases, and be willing to do whatever is necessary to achieve cultural competency. Skills must be integrated into education and training processes to prepare the laboratory professional to work effectively in and with different cultures.

INDEX TERMS: cultural competency; culture.


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The terms ‘majority’ and ‘minority’ will soon need to be reversed when used to refer to different groups within the total United States population. The group identified by the United States Census Bureau as white, non-Hispanic Caucasians, now the ‘majority’ or ‘mainstream’ culture, will soon become the minority group as ‘minority’ cultures continue to increase at a pace to outnumber the ‘majority’ groups in major urban areas. White, non-Hispanics currently comprise 73.6% of the total United States population, but this percentage will decrease to less than 53% by 2050. Hispanics and Asians are the two fastest growing population groups and are expected to comprise 24.5% and 8.2% respectively of the United States population at that time.

Culture is generally defined as a shared system of values, beliefs, traditions, and behaviors, as well as verbal and nonverbal patterns of communication that hold a group together and distinguish them from other groups; it shapes how we explain and value our world. Diverse is defined as describing anyone who is not you. The skill of using multiple cultural lenses is called cultural competence. Culture awareness implies recognition of culturally unique behaviors and cultural sensitivity implies a recognition and accommodation to culturally unique behaviors. Diversity is one of the defining characteristics of our age. Women, people of color, and immigrants, especially Asian and Latin Americans, are poised to constitute 80% of the workforce. Older workers, gays and lesbians, the disabled, and single parents will also assume greater positions of power in the future. Cultural competency incorporates cultural awareness, cultural sensitivity, and cultural appropriateness, but moves beyond requiring a mastery of cultural knowledge, perspectives, and behavior.

In the laboratory setting, the issues of workforce diversity and cultural competency become focused on how to help patients achieve healthy bodies. The framework for understanding cultural competence in healthcare delivery is based on the relationship between the patient and the healthcare professional, and the community and health system (Table 1).

PATIENTS AND CULTURAL COMPETENCY

Lack of awareness about cultural differences can make it difficult for both laboratory professionals and patients to achieve the best, most appropriate care. Fundamental differences among people arise from nationality, ethnicity, and culture as well as family back-
Patients from cultures in which stories or events are described by patients with linear time markers such as ‘first do, then do’, etc. is a particularly linear language. Americans give instructions to describe a sequence of events or conditions. English, for example, is responsible for thinking patterns and how people classify or process and what is important to members of a given culture. Language is cultural. Language development reflects the thought process and what is important to members of a given culture. It is also responsible for thinking patterns and how people classify or describe a sequence of events or conditions. English, for example, is a particularly linear language. Americans give instructions to patients with linear time markers such as ‘first do, then do’, etc. Patients from cultures in which stories or events are described by weaving in and out of a central theme or by starting from the conclusion and working inward in a circular sequence toward the initial event may have difficulty complying with English instructions, e.g., properly collecting a midstream urine specimen. Midstream urine instructions may require first a description of the type of specimen needed, i.e., not the first urine voided, but the urine that is voided after stopping urine flow, than starting it again, and why, followed by the steps given in reverse order. It could also require the laboratory practitioner to describe the specimen and relate its necessity to each step, e.g., clean the genital area so the specimen will contain only bacteria or ‘germs’ in the midstream urine and not those on the body or in the urine voided first; void some urine, stop, and void into the specimen cup because this midstream urine will have the bacteria found in the urine.

As patient populations become more diverse, basic communication with patients and families who speak little or no English is becoming more widely recognized as both a barrier to access to care and to the quality of care received. The ability to offer linguistically and culturally appropriate care is now a mandate in awarding Medicare contracts to managed care institutions and is now integrated into the Joint Commission on Accreditation of Health Care Organizations (JCAHO) accreditation process. Many facilities do not provide linguistically appropriate care, but rather rely on anyone on staff (whether professional or support) to act as interpreters. Rules of disclosure based on education, class, or status affect the patient’s disclosure of his or her need or the true nature of the complaint to someone of a much lower or higher status. Laboratory professionals need guidance on how to effectively utilize interpreters. Experienced interpreters listen for the meaning of the statement in its entirety and then translate that meaning into the second language.

Laboratory professionals need to know the relationship between ethnic background and expected test results. This requires knowledge of the cultures served in the community. Different laboratory test menus may be needed in different locales to properly serve the ethnic groups in the community. It is important that healthcare professionals respect the holistic view of health and illness, the importance of cultural forces, and the importance of spiritual beliefs. That would include, for example, respect for Jehovah’s Witness transfusion beliefs.

When an individual of one culture communicates something to someone from another culture, that message (verbal, non-verbal, or behavior) goes through a cultural rules filter that determines how the message is formulated. It is interpreted through the cultural filter of the receiver. These filters operate in both directions. The filters may lead to a misinterpretation of instructions, purposeful noncompliance with instructions, and/or dissatisfaction with the service received. The patient’s words or behaviors may also be misinterpreted. Mainstream Americans strongly believe in eye contact, but many other cultures consider it disrespectful to

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**Table 1. Basic components of cultural competence**

| Awareness, sensitivity, tolerance to cultural, language differences |
| Restraint from making judgments about beliefs, behaviors, needs, expectations of patients, colleagues |
| Understanding of culture and health/illness/prevention beliefs, practices |
| Recognition of impact of own culture, background on attitudes, beliefs, health, wellness, behavior, cleanliness, lifestyle |
| Knowledge of cultures of patient population served—taboos, health beliefs, rules of interaction |
| Skill to deliver culturally, linguistically appropriate patient advice, education |
| Effective utilization of interpreters |
| Culturally, linguistically appropriate interaction, delivery of care |
| Ability to deliver quality care to all cultures served |
look directly at persons in positions of authority such as a health (laboratory) professional.

Culture determines whether a person believes an illness was caused by fate, by God as punishment for a present or past sin, by a curse, by witchcraft, by jealousy, by germs, or by eating contaminated food. Culture dictates how laboratory professionals greet and address patients and how patients are expected to respond and vice versa. In the United States, there is an effort to demonstrate equality that equates with informality. This clashes radically with cultures that have carefully drawn hierarchies of authority or with cultures in which first names are rarely used by anyone other than a parent or spouse.

Mainstream American culture often demonstrates friendliness through touching and smiling. Many Native Americans will return smiles with cold, hard stares and not smile back. This is because their culture teaches them it is rude to smile at strangers. Many Asians recoil from touch while most Hispanic patients respond warmly to pats on the shoulder. Culture determines comfort with physical touch and the distance to be maintained between individuals. Laboratory professionals need to know what is acceptable for the patients served.

Laboratory professionals must deal with more subtle influences such as the patient’s perceptions of health, illness, and appropriate approaches to treatment. Clinical laboratory science curricula must be designed to enable students to learn about cultural competency and to be able to effectively partner with different cultures in healthcare decisions, and to function as their healthcare advocates.

New minorities will need the healthcare system in numbers disproportionate to their representation in the general population. The laboratory diagnoses and monitors many of the diseases prevalent in the growing minority populations (Table 2). Many minorities also have high risk diseases such as kidney disease and multi-system failure that result in outpatient care or hospitalization. An estimated 70% of clinical decisions are based on laboratory data.

Cultural competency means the ability and willingness to respond respectfully and effectively to people of all cultures, classes, races, ethnic backgrounds, and religions in a manner that recognizes and values the worth and dignity of all, whether similar to, or different from, the majority.

### IN THE WORK FORCE

The racial and ethnic makeup of America is changing and with it, the workplace environment. In the future, Hispanics will surpass African-Americans as the nation’s largest minority group. Caucasians are already the minority in California, Hawaii, New Mexico, and Washington DC, and will be in Texas before the decade ends. In the immediate future, 85% of the people entering the workforce will be women and minorities. These realities must be factored into laboratory career recruitment and into the workplace. Training and orientation of new employees need to be examined and updated appropriately. Changes will also be necessary on national and state certification and licensure examinations.

Laboratory management of a diverse staff with varied styles of communication, expectations, values, and experiences will present challenges. There are legal issues involved stemming from civil rights laws. Managers will be expected to assist all employees in reaching their full potential just as in a less complex ethnic environment. Every laboratory staff member will have to take time to accept and understand each other’s differences. Managers will have to commit to creating an environment in which that can happen.

Cultural synergy can provide growth and vitality to organization work teams and individuals. A facility or laboratory can improve market share and customer satisfaction by addressing special testing needs for the populations served and offering oral or written communication in another language, if indicated. Culturally insensitive practices can lead to patient dissatisfaction or defection of specific patient populations.

Ethnic resource guides for employees can be developed to teach and recognize the value of diversity in the community and in the laboratory. Such a document should include general background information and the common health practices of the cultures served. For example, some cultures have a high incidence of lactose intolerance or a higher incidence of certain abnormal hemoglobins. Also, dietary practices differ with cultures and some foods can affect laboratory test results.

A laboratory staff, or students, from different cultures, races, and religious backgrounds can present challenges for managers and educators. Laboratory professionals need to be challenged regarding cultural beliefs because some have no awareness of their own prejudices. Racial slurs or similar behavior cannot be tolerated and employees must be held accountable for such behaviors. Managers need to be sensitive to cultural cues such as eye contact and not hire or promote based on them.

Many laboratorians live on the ‘margins’ of society (Table 3). To be on the margin means not to be a part of the mainstream, popular

<table>
<thead>
<tr>
<th>Table 2. Diseases common in new minority populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
</tbody>
</table>
culture. In the United States, the popular culture or ideal for business success is young, white, heterosexual, Christian, and male.15

When a white female snubs an ethnic female, she is harming herself as well. The white female lives in the same margin as the ethnic female and is only perpetuating and cementing her place there. Different cultural groups have different values, styles, and personalities, each of which may have a substantial effect on how jobs are performed. Employers should recognize these differences as benefits and as a competitive edge in today’s marketplace.15

THE CHALLENGE

Laboratory professionals must become skilled in communication with all patient populations served. Each must be aware of his/her biases and be willing to do what is needed to achieve cross-cultural efficacy for all patients.3 Skills cannot be attained in a vacuum. They must be integrated into curricula throughout the laboratory education and training process. Educators and managers must be skilled and able to determine if employees and/or students are achieving cultural competence.

Cultural competence must be achieved to effectively work with both co-workers and patients being served. Laboratory professionals must know and understand the specific health risks for the populations served and understand their impact on laboratory testing: laboratory test menus should be appropriate for the patient populations in the community.

Laboratory professionals should understand and respect the co-worker cultures represented. A diversity of staff cultures should be viewed as beneficial to the work team rather than something that must be tolerated. The laboratory must become color blind to effectively meet the needs of all people accessing and working in the healthcare system. Cultural sensitivity should permeate every laboratory and healthcare system.

REFERENCES


Table 3. Individuals on the margin

<table>
<thead>
<tr>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ethnic background that is non-white</td>
</tr>
<tr>
<td>Not heterosexual</td>
</tr>
<tr>
<td>Not Christian</td>
</tr>
<tr>
<td>Not between the ages of 21 and 50</td>
</tr>
</tbody>
</table>
The hospital transfusion services received an order for three units of red blood cells for a 45-year-old Caucasian male. His most recent hematological study revealed a hemoglobin level of 7.0 g/dL (Table 1). Blood was obtained from the patient and the clinical laboratory scientist (CLS) proceeded with pre-transfusion testing of phenotype and antibody screening. The results of the forward typing of ABO and Rh indicated that the patient was phenotype A Rh positive (Table 2). However, a discrepancy was found in the reverse typing with A1 cells and B cells producing agglutination. Using antibody-screening cells, the patient’s serum was tested. The results indicated agglutination upon immediate spin, but no agglutination in subsequent testing including 37 °C with LISS enhancement (Table 3).

The CLS requested additional history on the patient. The diagnosis was multiple myeloma; there had been no previous transfusions.

**DISCUSSION**

Multiple myeloma is a hematological malignant neoplasm of the bone marrow. It is a neoplastic disease characterized by the infiltration of bone and bone marrow by myeloma cells forming multiple tumor masses. Production of normal immunoglobulins is impaired with a significant increase in the number of abnormal plasma cells. The condition is usually progressive and generally fatal. The disease causes pain, fractures, anemia, hypercalcemia, kidney failure, bacterial infections, nerve compression with paralysis, skeletal deformities, and changes in mental status ranging from mild to severe confusion. According to the American Cancer Society, about 14,400 new cases will be diagnosed and about 11,200 Americans are expected to die of multiple myeloma in 2001.

**Etiology and epidemiology**

The etiology of multiple myeloma is unknown; however, genetics, radiation exposure and chronic antigenic stimulation have been suggested as predisposing factors. Increases in the incidence of multiple myeloma during this past century implicate environmental factors as important causal agents. A single insult is not thought to be sufficient to induce the disease. However, continued exposure results in the clonal expansion of an idiotypic plasma cell after cumulative mutational damage has altered its genetic makeup. Atomic bomb survivors and individuals exposed to radiation in the workplace have demonstrated an increased incidence of mul-

<table>
<thead>
<tr>
<th>Table 1: Hematological profile</th>
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<tbody>
<tr>
<td><strong>Analyze</strong></td>
</tr>
<tr>
<td>RBC</td>
</tr>
<tr>
<td>WBC</td>
</tr>
<tr>
<td>HGB</td>
</tr>
<tr>
<td>HCT</td>
</tr>
<tr>
<td>MCV</td>
</tr>
<tr>
<td>MCH</td>
</tr>
<tr>
<td>MCHC</td>
</tr>
<tr>
<td>Platelets</td>
</tr>
</tbody>
</table>

Peripheral smear marked rouleaux no rouleaux
tiple myeloma. Studies of workers at nuclear power plants also suggest that chronic exposure to low levels of radiation may lead to increased risk. The molecular and cytogenetics of cells in multiple myeloma are under investigation, but the precise causes of these abnormalities are largely unknown.

Chronic stimulation of the immune system has been a suspected trigger of multiple myeloma with certain medical conditions such as rheumatoid arthritis, chronic allergic conditions, and chronic infections as they are implicated in the stimulation of the aberrant production of plasma cells. Anticipation, a phenomenon in which an inherited disease is diagnosed at an earlier age in each successive generation of a family, has been demonstrated in multiple myeloma.

The incidence of myeloma is five cases per 100,000 persons each year and males have approximately 50% greater risk than females. There is greater incidence in black individuals than white individuals; persons of Japanese and Chinese descent experience the least incidence. Age increases the risk of multiple myeloma as the disease is rarely seen in persons less than 40 years of age with the mean onset at age 60.

**CLINICAL PRESENTATION AND PATHOPHYSIOLOGY**

Multiple myeloma does not have the same biology in all patients; it is best viewed as a heterogeneous disease with different prognoses, clinical course, and response to therapeutic interventions in different subjects.

Patients may present with persistent unexplained skeletal pain (usually in the back or thorax), weakness and fatigue, confusion, and/or recurrent bacterial infections. This disease is diagnosed in some patients with no symptoms after a screening blood test reveals abnormally high serum protein levels or there is evidence of calcium loss from bones. In some cases, patients are diagnosed only after they have developed marked changes in their mental state with extensive bone destruction and kidney failure. Pathological fractures and vertebral collapse are common. Renal failure may be caused by extensive cast formation in the renal tubules, atrophy of tubular epithelial cells, and interstitial fibrosis. Anemia predominates in some patients, and a few have manifestations of hyperviscosity syndrome.

An expanding plasma cell mass in the bone marrow undergoes continued clonal replication. As the growth proceeds, normal bone marrow is gradually replaced by the steadily growing malignant plasma cell colonies. Normal circulating blood cells decrease in number resulting in anemia, thrombocytopenia, and neutropenia. The resulting pancytopenia causes fatigue, delayed hemostasis, and an increased susceptibility to bacterial infections.

The expanding plasma cells infiltrate bone-causing destruction of the surface cortex of the bone. Stretching of the overlying nerve-rich periosteum leads to pain that is present at diagnosis in more than two-thirds of patients. The destruction of bone tissue results in an increase in blood calcium levels (hypercalcemia). Diffuse osteoporosis or discrete osteolytic lesions develop, usually in the pelvis, spine, ribs, and skull. Lesions are due to bone replacement by expanding plasmocytomas or a factor secreted by malignant plasma cells (osteoclast-activating factor). The osteolytic lesions are usually multiple, but occasionally are solitary intramedullary masses. Extra-osseous plasmacytomas are unusual, but may occur in any organ, especially the upper respiratory tract.

Hypercalcemia occurs in 15% of patients with multiple myeloma at diagnosis and should be suspected in the presence of anorexia, nausea, vomiting, polyuria, polydipsia, increased constipation, weakness, confusion, or stupor. Because calcium affects nerve cell function, hypercalcemia can cause weakness and confusion. If hypercalcemia is untreated, renal insufficiency develops as well.

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**Table 2. Initial patient ABO and Rh phenotyping reactions**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>4+</td>
</tr>
<tr>
<td>Anti-B</td>
<td>0</td>
</tr>
<tr>
<td>Anti-D</td>
<td>3+</td>
</tr>
<tr>
<td>Rh Control</td>
<td>0</td>
</tr>
<tr>
<td>A₁ cells</td>
<td>1+</td>
</tr>
<tr>
<td>B cells</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Table 3. Antibody screening and initial crossmatch results**

<table>
<thead>
<tr>
<th></th>
<th>Immediate Spin</th>
<th>37 °C/LISS enhancement</th>
<th>AHG</th>
<th>AHG control cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening Cell I</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Screening Cell II</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Screening Cell III</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Auto Control</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
</tbody>
</table>
The malignant plasma cells produce immunoglobulins resulting in an overproduction of intact immunoglobulins (IgG, IgA, IgD, or IgE) or Bence Jones protein. Plasmacytomas produce IgG in about 55% of myeloma patients and IgA in about 20%; of these IgG and IgA patients, 40% also have Bence Jones proteinuria. Light chain myeloma is found in 15% to 20% of patients; their plasma cells secrete only free monoclonal light chains, and a monoclonal spike is usually absent on serum electrophoresis.1

Laboratory findings and diagnosis
During the different stages of the disease, almost all patients develop anemia usually presenting with a normocytic normochromic anemia with hemoglobin levels between 7.0 and 12.0 g/dL.9 The peripheral smear shows rouleaux formation as the result of elevated globulins or fibrinogen in the plasma.12 Red cells that are constantly bathed in the abnormal plasma affect a spontaneous pseudo-agglutination which appears as stacks of coins in the peripheral smear.13 These stacks appear evenly dispersed throughout the smear. Rouleaux formation correlates with a high erythrocyte sedimentation rate and occurs as a direct result of protein deposition on the erythrocyte membrane.14

Large amounts of protein can cause the peripheral blood smear to have a bluish tinge macroscopically. A few abnormal plasma cells may be seen in later stages on the peripheral blood differential. The leukocyte and platelet counts usually are normal in early stages of the disease until overpopulation of the marrow with abnormal plasma cells occurs. This may produce pancytopenia and elicit a leukoerythroblast response.11 Serum creatinine, BUN, LDH, calcium, protein, and serum uric acid are frequently elevated. Monoclonal peaks of immunoglobulin can be found in serum protein electrophoresis. The immunoglobulin type can be determined by immunoelectrophoresis or immunofixation electrophoresis. Bence-Jones protein or light chain proteins can be identified in urine in 80% of myeloma patients.12

Bone marrow aspiration and biopsy usually indicate increased numbers of plasma cells at various stages of maturation. Rarely is the number of plasma cells normal; and usually more than 10% and often more than 30% of total bone marrow cells are present. Diagnostic criteria for multiple myeloma is based on a combination of the presence of multiple criteria including plasmacytoma, greater than 30% plasma cells in the bone marrow, multiple lytic bone lesions, monoclonal protein spike in serum protein electrophoresis, and depressed synthesis of normal immunoglobulins.5 Laboratory findings that may occur in multiple myeloma are provided in Table 4.

Treatment and prognosis
The disease is progressive, but good management improves quality and duration of life. Prognosis varies and is dependent on the stage of the disease at diagnosis. The median survival rate is two to three years. The patient with multiple myeloma should be carefully evaluated from the standpoint of symptoms, physical findings, and laboratory data. If there are no symptoms or evidence of early or impending complications, treatment is often delayed until progression of the disease occurs. About 60% of patients treated show objective improvement. At diagnosis, high levels of monoclonal protein in serum or urine, elevated beta-2-microglobulin levels, diffuse bone lesions, hypercalcemia, anemia, and renal failure are unfavorable prognostic signs.3

Chemotherapy is helpful in prolonging survival. Median survival of chemotherapy nonresponders is less than one year and responders three to four years.7 A cure is not presently attainable with standard chemotherapy, interferon, or high-dose chemotherapy followed by autologous transplantation regimens with bone marrow.

**Table 4. Possible laboratory findings that may occur in multiple myeloma**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Possible findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum chemistries</strong></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>elevated</td>
</tr>
<tr>
<td>Calcium</td>
<td>elevated</td>
</tr>
<tr>
<td>BUN</td>
<td>elevated</td>
</tr>
<tr>
<td>Creatinine</td>
<td>elevated</td>
</tr>
<tr>
<td>LDH</td>
<td>elevated</td>
</tr>
<tr>
<td>Uric acid</td>
<td>elevated</td>
</tr>
<tr>
<td><strong>Immunological studies</strong></td>
<td>decreased</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>monoclonal</td>
</tr>
<tr>
<td>SPE</td>
<td>gammopathy</td>
</tr>
<tr>
<td>C-Reactive protein</td>
<td>positive</td>
</tr>
<tr>
<td><strong>Urine chemistries</strong></td>
<td></td>
</tr>
<tr>
<td>Bence-Jones Protein</td>
<td>present</td>
</tr>
<tr>
<td>Protein</td>
<td>present</td>
</tr>
<tr>
<td><strong>Hematological studies</strong></td>
<td>elevated</td>
</tr>
<tr>
<td>ESR</td>
<td>decreased</td>
</tr>
<tr>
<td>WBC</td>
<td>decreased</td>
</tr>
<tr>
<td>RBC</td>
<td>decreased</td>
</tr>
<tr>
<td>Platelets</td>
<td>decreased</td>
</tr>
<tr>
<td><strong>Peripheral smear</strong></td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>present in</td>
</tr>
<tr>
<td>Rouleaux</td>
<td>advanced disease</td>
</tr>
<tr>
<td>Hemostasis</td>
<td></td>
</tr>
<tr>
<td>APTT</td>
<td>prolonged</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>prolonged</td>
</tr>
</tbody>
</table>
or peripheral blood stem cells. Cure or long-term disease-free survival is seen in less than 20% of patients under 55 years of age who have related-donor match and receive an allogeneic graft, either from bone marrow or peripheral blood stem cells. Treatment-related mortality for patients treated with this approach still exceeds 50%. If the patient is younger than 70 years, autologous peripheral blood stem cell transplantation is considered. If the patient is older than 70 years, chemotherapy is indicated. Chemotherapy decreases serum or urine monoclonal protein and increases median survival time three to sevenfold.

Because anemia occurs in almost all patients during the course of multiple myeloma, transfusion of packed RBCs is indicated for symptomatic anemia. In recent prospective, randomized, placebo-controlled blind clinical trials, it has been demonstrated that erythropoietin/epoetin alfa is beneficial in the treatment of anemia in multiple myeloma as well.

ABO discrepancies

ABO discrepancies occur when the red cell testing does not agree with the expected serum testing. Washing the patient red blood cells with saline can usually resolve the ABO discrepancy if the initial test was performed using red blood cells suspended in serum or plasma. ABO discrepancies are divided into four groups:

- **Group I** are discrepancies between forward and reverse groupings because of weakly reacting or missing antibodies.
- **Group II** are discrepancies between forward and reverse groupings resulting from weakly reacting or missing antigens.
- **Group III** discrepancies are found between forward and reverse groupings caused by protein or plasma abnormalities and result in rouleaux formation or pseudo-agglutination.
- **Group IV** discrepancies are between forward and reverse groupings encompassing miscellaneous problems such as warm or cold autoantibodies and polyagglutination.

Multiple myeloma elevates the globulin level resulting in rouleaux and Group III ABO discrepancies. Because rouleaux formation causes the red blood cells to adhere to one another as in stacked coins, it can be mistaken for agglutination by a new or inexperienced laboratorian. Phenotyping can usually be accomplished by washing the patient’s red cells several times with a saline solution. Using washing techniques as outlined in the American Association of Blood Banks Technical Manual, serum is removed from the centrifuged serum/cell mixture. The cells are then resuspended in saline and recentrifuged. This saline is removed from the cell button, fresh saline is added, and the cells are resuspended once again. This is repeated three times. The washed cells are used for testing. This washing technique rids the red cell membranes from the protein and frees the cells in the case of rouleaux formation in the reverse type. In true agglutination, red cells will continue to clump after the washing with saline.

**CASE RESOLUTION**

This patient was diagnosed with multiple myeloma six months prior to his admission to the hospital and the transfusion order. The positive patient control cells (Table 3) indicate that something is coating the patient’s red cells. The patient’s red cells were washed with normal saline after complete decanting of the serum. After repeating the forward and reverse typing with the washed cells, the patient’s phenotype was confirmed to be A positive. The rouleaux disappeared from all phases of the testing. The CLS completed the crossmatch and the three units of A positive blood were successfully transfused.

**REFERENCES**

Parvo B19 (Fifth disease) is an erythrotropic virus which attaches through the ‘P’ globoside receptor on the surface of human red blood cells and precursors. This typically benign viral infection can cause a transient aplastic anemia in patients with underlying red cell disorders. In this case, a two-year-old child presents with severe aplastic anemia without evidence of underlying disease. Erythroid regeneration is monitored through the use of the immature reticulocyte fraction (IRF) and is demonstrated by the presence of high and medium fluorescence reticulocytes in the peripheral blood three to five days prior to the peak in absolute reticulocytes.

**ABBREVIATIONS:** IRF = immature reticulocyte fraction.

**INDEX TERMS:** erythroid hypoplasia; erythroid marrow regeneration; flow cytometry; globoside-p antigen; hemolytic anemia; parvovirus B19 infection; reticulocytes.


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Parvovirus B19 (B19) is a species specific, small, nonenveloped, single-stranded DNA virus that belongs to the family Paroviridae, genus *Erythroivirus*. Discovered by Cossart and colleagues in 1975, it is the only known human pathologic parvovirus associated with a broad spectrum of disorders. Human B19 is considered a ubiquitous virus with distribution worldwide. Cultured from the respiratory tract, Parvovirus B19 is presumed to be transmitted as an aerosol. Prevalence of infection is common, reported at a frequency ranging from 2% to 15% in children one to five years of age, approximately 15% in children ages five to 19 years, with approximately 50% of adults testing seropositive. Diverse clinical manifestations arise due to B19 infection. Infection follows a cyclic pattern with increased rates occurring every four to five years. Infection can present as asymptomatic, cause *erythema infectiosum,* or induce a polyarthropathy syndrome, a hemolytic anemia, or hydrops fetalis during pregnancy. Severe anemia due to bone marrow aplasia is often a major complication as a result of viral replication in erythroid precursors. The most common clinical manifestation, *Erythema infectiosum* or Fifth disease is a benign, self-limiting febrile illness associated with marked erythema of the cheeks, known as ‘slapped cheek’, and a lacy rash on the trunk and extremities. A migratory polyarthropathy syndrome, immunologically mediated, may occur in infected children and adults, particularly women, with symptoms generally persisting for one to three weeks. By the time the rash or polyarthropathy presents in immunocompetent patients, viremia has cleared and the presence of serum antibodies confirms the diagnosis. Studies have shown that patients with an underlying chronic hematologic disease, such as sickle cell anemia, or those with immunodeficiency states, such as HIV, are at risk for severe anemia with transient aplastic crisis of the bone marrow.

Parvovirus B19 has a predilection for replication in the bone marrow erythroid progenitor cells including the erythroid colony-forming units (CFU-E) and the burst-forming units (BFU-E). Infection suppresses erythropoietic activity of the bone marrow. The B19 virion consists of capsid proteins and DNA genome. The capsid proteins give stability to the B19 virus. Studies have shown that the P blood group antigen, a globoside, serves as the receptor for B19 capsid attachment. Globoside is found on cells within the bone marrow such as early erythroid progenitor cells, megakaryocytes, and on cells within the placenta, fetal myocardium, kidney, and thyroid. Myocarditis contributes to the hydrops fetalis associated with B19 infection. Studies have shown that rare individuals who lack the P antigen and therefore lack the receptor for the virus are not susceptible to B19 infection. Viral replication, by means of host cellular DNA polymerase, occurs within the nucleus of actively dividing cells, following the S phase of the host’s cell cycle. The palindromic sequences of the genome enables the molecule to fold over on itself and form a hairpin structure important for replication. The 5 kb genome of the human B19 parvovirus...
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encodes for structural proteins VP1 and VP2 and the non-structural proteins NS1 and NS2. The infection of susceptible cells and the expression of non-structural protein NS1 has been shown to impair cellular mechanisms and promote apoptosis of erythroid progenitors. Cell death can occur without viral replication because the production of the nonstructural proteins are cytotoxic. Immunologic suppression of bone marrow erythropoiesis has also been reported to occur associated with B19 infection. Individuals at risk for prolonged B19 infections are those who do not develop neutralizing antibodies to VP1 as measured in erythroid progenitor assays.

A hemolytic anemia associated with transient bone marrow erythropoiesis is a serious complication of Parvovirus B19 infection. Infected cells fail to proliferate and mature thus prohibiting the production of new red blood cells. Bone marrow examination reveals dyspoietic changes to pronormoblasts with few mature normoblasts present. Reticulocytopenia is the cardinal sign of an aplastic crisis.

Disturbances in the dynamic equilibrium of erythropoiesis can be monitored clinically by the absolute reticulocyte count and the maturity of the subpopulations of reticulocytes quantified by flow cytometry. Red blood cell survival studies in normal individuals show the lifespan of a reticulocyte to be one to two days in peripheral circulation. During periods of increased erythropoiesis, usually accompanied by increases in erythropoietin, the lifespan of a reticulocyte in circulation is increased to three or more days, owing to the release of ‘stress or shift’ reticulocytes from the bone marrow and accelerated erythroid differentiation. The quantity of RNA found in reticulocytes is a measurable biochemical change within the cell that can define the maturity of the various subpopulations in circulation. Bone marrow ‘stress or shift’ reticulocytes are primarily defined by the high amount of RNA found within the cell. Thus, the ability to not only count but to fractionate reticulocyte populations based on RNA content into levels of maturity has significant clinical application in assessing erythropoietic activity.

The Sysmex R-series is a fully automated, multiparameter flow cytometer that utilizes light scatter and the fluorescent dye Auramine-O (AuO) to bind in a stoichiometric fashion to the RNA within the reticulocyte (Sysmex Corporation, Gilmer Road 6699, Long Grove, IL 660047, a subsidiary of TOA Medical Electronics Co., LTD, 1-800-3-SYSEMEX, www.sysmex.com). The use of this methodology permits reticulocytes to be counted and discriminated based on the content of RNA in the cell. Fluorescence is divided into three regions known as Low Fluorescent Reticulocytes (LFR), Medium Fluorescent Reticulocytes (MFR), and High Fluorescent Reticulocytes (HFR). Highly fluorescent reticulocytes contain the most cellular RNA and represent those reticulocytes recently released from the bone marrow into the peripheral circulation. The quantification of RNA in reticulocytes has generated a useful clinical parameter known as the immature reticulocyte fraction or IRF. This parameter has significant clinical application in assessing regenerative and nonregenerative hematologic conditions. The IRF is defined as the sum of the HFR and MFR region and as such is the standard nomenclature recommended by NCCLS guidelines. In the Sysmex R series analyzers, the sum of HFR and MRF demonstrates an asymmetric log-normal distribution, thus making an increased IRF value of clinical significance. Studies have demonstrated that the IRF of bone marrow is consistently higher than that of peripheral blood with a mean of 0.41 versus 0.34 respectively, owing to the shift toward the more immature and RNA rich proportion of reticulocytes in the bone marrow. Bivariant analysis of the IRF and the absolute reticulocyte count provides data to assess the adequacy of the erythropoietic response for the level of anemia as well as a classification scheme for anemia as demonstrated in various studies. The presence of both a low absolute reticulocyte count and a low IRF suggests a reduced level of erythropoiesis associated with aplastic or hypoplastic phases. Increased erythropoietic activity following an aplastic crisis generally manifests with normal to increased IRF with below normal reticulocyte counts. Patients with a compensated hemolytic anemia present with increases in both the reticulocyte count and the IRF.

CASE PRESENTATION

A 25-month-old male Caucasian child was brought to the emergency room by his mother. Physical examination revealed an acholic, pale, lethargic child. Skin and mucous membranes were pale and gums blanched. Clinical history revealed a mild flu-like illness accompanied by a low-grade fever over the past week. During this time, the child had been in day-care for three days. There had been no major illnesses nor were there any underlying disorders noted. All immunizations were complete and up-to-date. The child appeared of average weight and size for his age. The patient was admitted to the hospital the same day. Complete blood and chemistry profiles were ordered.

Laboratory results

On admission, results were as follows: WBC 6.9 x 10^9/L; RBC 1.26 x 10^{12}/L; hemoglobin 3.9 gm/dL; hematocrit 11.8 %, and absolute reticulocytes 19.0 x 10^9/L (Table 1). Values between 10 and 110 x 10^9/L are considered as the normal absolute reticulocyte reference range according to NCCLS Standards. The differential showed 50 PMNs, 7 bands, 37 lymphs, 5 monocytes, and 1 metamyelocyte. Serum chemistries were essentially normal with the exception of total bilirubin and LD (Table 2). Blood bank results showed the patient to be group ‘O’ with a positive DAT, all cells positive on antibody screen (with IgG Coombs), and an inconclusive Rh typing. A unit of packed red blood cells aliquoted into two bags was ordered for transfusion. The patient was transfused following a biologic crossmatch in an attempt to maintain hemoglobin and hematocrit levels. Additional laboratory tests ordered included: urinalysis, CMV cultures, hepatitis profile, and serologic tests for CMV and Parvo B19.

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Subsequent to admission, the child was hospitalized for 15 days, released, and followed at the clinic during which time the following pertinent laboratory data was compiled (Tables 1, 2, and 3). During the second week of hospitalization, the child presented with a pronounced erythematous rash primarily involving the cheeks of the face, upper trunk, arms, and legs. In the two-day period following admission and in vivo crossmatch, the patient’s red cell counts, hemoglobin, hematocrit, and absolute reticulocytes continued to fall (Tables 1 and 3). Serum chemistries showed significant elevations in lactate dehydrogenase (LD) and total bilirubin, with alterations in the total protein, albumin and globulin fractions (Table 2). Rises in LD and total bilirubin with a continual drop in the hemoglobin and hematocrit were consistent with a noncompensated hemolytic crisis. Urinalysis results were unremarkable except for the presence of two WBCs per high power field. Results on initial blood and urine samples for CMV were negative.

Noting all major abnormal laboratory findings, a presumptive diagnosis of immune mediated hemolysis accompanying Fifth’s disease was made. An eluate was performed with all panel cells reactive with IgG Coombs. A second biologic crossmatch was performed and a second unit of packed red cells was issued and transfused on day four following admission. Following the second transfusion of packed red cells, post-transfusion rises in the hemoglobin and hematocrit were achieved. LD and total protein continued to rise however, reaching 1566 IU/L, and 8.9 mg/dL respectively on day ten following admission and red cell transfusion. Total bilirubin decreased significantly following admission and remained mildly elevated throughout hospitalization (Table 2).

An absolute peripheral blood reticulocytopenia classically defining bone marrow erythroid hypoplasia ensued for six days as documented by the absolute reticulocyte count (Table 3).

A blood specimen was analyzed for Parvovirus B19 antibody 23 days following admission and presentation to the clinician. Testing revealed IgM levels of 2.58, and IgG levels of 2.74; values >1.20 are interpreted as positive. Both IgM and IgG were positive, consistent with current or recent infection within the last two to three months. A follow-up specimen drawn approximately two weeks after the first specimen tested below threshold values for Parvo B19 IgM (1.13) but positive for Parvo B19 IgG (2.26).

The decrease of IgM antibody and the persistence of IgG antibody correlates with recovery from recent infection and immunity. In general, viremia precedes the onset of Fifth

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<tr>
<td>HGB (gm/dL)</td>
</tr>
<tr>
<td>HCT (%)</td>
</tr>
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<td>RETIC‡ (%)</td>
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* (a) pre-transfusion results; (b) post-transfusion
† Day of discharge
‡ RBC and reticulocyte counts taken from Sysmex Series

<table>
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<th>Table 2. Chemistry results during hospitalization and clinic follow-up</th>
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<td>T Protein (mg/dL)</td>
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<tr>
<td>Albumin (gm/dL)</td>
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<tr>
<td>Globulin (gm/dL)</td>
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</table>

* Weeks after discharge
disease and the associated transient aplastic crisis. Although no actual viral serology was done during the acute phase of the disease, the globulin level on day five was significantly elevated (5.5 gm/dL), most likely indicative of a higher antibody titer. Blood bank results support the evidence of an immune response as seen by the positive DAT on admission and a positive IgG eluate. Following the peak in globulin on day five, recovery of bone marrow reticulocytes is evident by way of increasing absolute reticulocyte counts (Table 3). The assumption is that viral particles and infected cells are now being cleared from the system thus allowing bone marrow regeneration of reticulocytes and maintenance of the peripheral blood red cell count (Table 3; Figure 1). Review of the peripheral blood differential indicated an absolute lymphocytosis concurrent with the globulin peak.

Hematologic findings in this pediatric patient demonstrated an immune hemolytic anemia with an aplastic crisis of the bone marrow as evidenced by the use of the IRF and the absolute reticulocyte count (Table 3; Figure 1). The IRF directly assesses reticulocyte maturity; the absolute reticulocyte count accounts for the severity of the anemia; and the combined use evaluates the adequacy of the marrow erythropoietic response. In general, it has been noted that patients with a severe anemia or significant reticulocytosis as defined by increases in absolute reticulocyte count also had increased IRF. Hypoplastic or aplastic crisis are defined by decreases in both the absolute reticulocyte count and the IRF. Sequential measurements of the IRF and absolute reticulocyte counts in bone marrow transplant patients demonstrate a reproducible pattern highlighting early signs of erythroid regeneration within the bone marrow.

### DISCUSSION

The diagnosis of human Parvovirus is generally determined by the serologic presence of IgM and/or IgG antibodies and a concomitant hemolytic anemia as demonstrated by decreasing hemoglobin and absolute reticulocyte counts. In general, transient aplastic crises are common often requiring supportive care and transfusion. Pregnant women, immunocompromised individuals, and those with chronic hemolytic anemias are at the highest risk for complicated parvovirus infection. Viral titers are usually elevated during clinical manifestations of the disorder. IgM antibody against the virus rises and generally peaks within 14 days after the onset of the disease followed by a rise in the IgG antibody. Immunity is considered lifelong following infection.

Bone marrow infection manifests by a transient erythroid hypoplasia which morphologically presents as dysplastic, megaloblastic pronormoblasts with intra-nuclear bodies. Parvovirus B19 has a strong tropism for, and cytotoxic properties against, erythroid progenitors preventing cellular replication and maturation. Studies demonstrate that a non-structural gene protein promotes apoptosis of the infected erythroid cell line. The P antigen, a globoside found on erythroid progenitor cells, serves as cell receptor for viral attachment.

The associated hemolytic anemia and transient aplastic crisis has classically been monitored by the peripheral reticulocyte count reported as either the percent reticulocytes or absolute reticulocyte counts (10^9/L). The IRF has been shown to increase three to five days prior to the increase seen in reticulocyte counts and therefore can assess marrow erythropoietic activity earlier than the reticulocyte count. The IRF is an early and sensitive indicator of erythropoiesis. Those patients with a severe degree of anemia are expected to demonstrate an increased IRF with decreased absolute reticulocyte counts, indicative of the expected physiologic response of increased erythropoiesis. In patients with a chronic hemolytic anemia...
mia, there is an increase in the IRF as well as an absolute reticulocytosis. Severe hypoplasia results in a decrease in both the IRF and the absolute reticulocyte count. Bivariant analysis of the absolute reticulocyte numbers and the IRF aid to further define the adequacy of the response.

Serial measurements of the IRF has practical significance in its ability to assess the status of engraftment during the post-transplant period. Early studies show that the absolute reticulocyte counts and the reticulocyte percent were insensitive in predicting engraftment. The first response following bone marrow ablation is an increase in the IRF which generally precedes the increase in the reticulocyte count. With the increased sensitivity and clinical utility of the IRF, a better means of monitoring the regenerative bone marrow response following an aplastic crisis or stem cell transplantation has evolved.

Clinical utility of the IRF not only has application in the evaluation and classification of anemia as a result of erythroid hypoplasia, but in determining therapeutic protocol and efficacy of erythropoietic treatment in chronic renal failure and in assessing hematopoietic regeneration following chemotherapy, radiation, or bone marrow or peripheral blood stem cell transplantation. The IRF also has use in monitoring the therapeutic response to iron, folic acid, vitamin B12, and is useful in redefining apheresis protocols when harvesting peripheral stem cells.

**Figure 1.** Comparison of absolute reticulocytes vs. fluorescent reticulocyte fractions

Fluorescent reticulocyte (%) counts taken from Sysmex Series Analyzer
- HFR = high
- MFR = medium
- LFR = low

**REFERENCES**
HIV-1 Antiretroviral Resistance Testing Laboratories

LOUISE K HOFHERR, DIANE P FRANCIS, J REX ASTLES, WILLIAM O SCHALLA

OBJECTIVE: To identify and to describe the genotyping and the phenotyping testing practices of U.S. laboratories performing patient HIV-1 antiretroviral resistance testing.

DESIGN: A self-report 44-item mailed questionnaire.

PARTICIPANTS: Laboratories potentially performing HIV-1 antiretroviral resistance testing.

MAIN OUTCOME MEASURE: Descriptive study.

RESULTS: Of 236 laboratories surveyed, 165 (69.9%) returned completed surveys, but only 23 performed HIV-1 antiretroviral resistance testing. Most were university hospitals (47.8%) or independent laboratories (26.1%). All 23 laboratories used genotypic methods, while nine (39.1%) used both genotyping and phenotyping. Most testing was used for clinical trials or laboratory research. The amount of patient information collected by laboratories varied, as did their type of quality assurance measures. Variation was found with regard to: testing volume, testing experience, testing reasons, testing methods availability, testing controls, specimen treatment, and storage stability.

CONCLUSIONS: Due to variation in practices in this area of patient testing, it may be advantageous for laboratory professionals to reach a consensus on what is the most acceptable.

ABBREVIATIONS: 3TC = lamivudine; AZT = zidovudine; CDC = Centers for Disease Control and Prevention; CLIA = Clinical Laboratory Improvement Amendments; CT = clinical trials; d4T = stavudine; ddl = didanosine; DLV = delavirdine; DT = drug treatment; ENVA = Evaluation of New Antiviral Treatments; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; LAP = Laboratory Assurance Program; LR = laboratory research; MPEP = Model Performance Evaluation Program; NVP = nevirapine; PT = patient treatment; QA = quality assurance; QC = quality control; VL = viral load.

INDEX TERMS: clinical laboratory techniques; drug resistance; HIV-1; quality control; viral RNA.


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In recent years, the development of antiretrovirals, including the use of monotherapy, and the discovery of the synergistic effects of antiretroviral drug combinations in reducing virus population, have impacted disease progression in those infected with human immunodeficiency virus (HIV). Along with these developments, tremendous strides have been made in our knowledge of the pathogenicity of HIV.1-4 Discovery of the massive amounts of virus production and huge viral turnover with high mutation rates offer tremendous challenges. One of these is to reduce viral replication and the subsequent mutation rates, estimated to be $3 \times 10^{-5}$ mutations per nucleotide per replication, which would result in more effective antiretroviral therapy.5 As new agents are developed and current ones are increasingly used, it is vitally important to determine when they become ineffective in order to modify therapy. Antiretroviral resistance testing provides key information for monitoring treatment efficacy.

Highly active antiretroviral therapy (HAART) is currently considered the optimal treatment for HIV-1 infection.6 The goal of this therapy is to minimize virus levels and suppress viral replication. For successful treatment using HAART, identifying the development of mutations that confer resistance is vital. As the HAART protocol becomes more common, as the number of infected individuals who meet the criteria to initiate antiretroviral therapy increases, and as the number of antiretroviral agents continues to expand, it is expected that laboratory testing for HIV-1 antiretroviral resistance will continue to increase.

Clinical laboratory practices in this area are extremely heterogeneous and lack standardization. To our knowledge, no large-scale, systematic study has occurred in the area of the phenotyping and genotyping methods or practices among laboratories performing HIV-1 antiretroviral resistance testing. Such a study is necessary in determining what measures might be taken to standardize methods and practices among laboratories in the U.S. so that patients and their physicians could obtain comparable results.
To this end, in 1999 the Laboratory Assurance Program (LAP) at San Diego State University in cooperation with the Division of Laboratory Systems, Public Health Practice Program Office, Centers for Disease Control and Prevention (CDC) undertook a survey to determine the number of laboratories performing antiretroviral resistance testing, and the methods they use.

METHODS

Survey Population

Names of laboratories potentially performing viral resistance testing came from many sources. These included: 1) CDC’s Model Performance Evaluation Program (MPEP) list, containing HIV-1 RNA testing laboratories that participate in CDC’s MPEP; 2) Association for Molecular Pathology 1998 Test Directory; 3) laboratories participating in the Evaluation of New Antiviral Treatments (ENVA) conducted by Dr. Rob Schuurman of the University Hospital Utrecht, Utrecht, The Netherlands; 4) personal communication with experts in this area; 5) prior information from LAP surveys; and 6) flyers distributed at two national conferences (14th Annual Conference on Human Retrovirus Testing, Association of Public Health Laboratories, Albuquerque, NM, March 1999; Second Annual Conference on AIDS Research in California, San Francisco, CA, February 1999).7

The survey consisted of a 44-item, eight-page questionnaire, which queried laboratories about their HIV-1 antiretroviral resistance testing practices. Items were also included which described each laboratory. Both closed- and open-ended questions were used. The questionnaire was designed with help from experts in the field of antiretroviral resistance testing. The experts consisted of one MD and five PhDs who perform and conduct research in antiretroviral resistance testing, and who in some cases authored papers in this field in peer-reviewed journals. The experts received a draft of the survey questionnaire to critique and suggested changes either by mail or telephone. The final survey reflected their comments and suggestions.

Each survey packet mailed to participants included a cover letter, the questionnaire, a business reply envelope, and a brochure describing the MPEP provided by CDC. The survey was administered utilizing a three wave system. The first wave was mailed in July 1999. Nonresponders subsequently received the second wave in August 1999, and those still nonresponsive received the third wave in October 1999. The survey was closed in December 1999.

Data Management and Analysis

Graduate students in public health, using Paradox (Paradox for Windows, Version 5.0, Borland International, Inc. 1994) entered the questionnaire data into a database. When completed, a random sample of returned questionnaires was checked to ensure accuracy of data entry. The statistical package SPSS (SPSS for Windows, Version 8.0, Inc. 1997) was used for all data editing and analysis.

Frequencies were calculated for all variables, and stratification was carried out for certain variables to determine their impact on the distributions described. Data were stratified by laboratory type, testing methodology, CLIA certification, and purpose of testing. In some cases, stratification resulted in numbers too small to be meaningful.

RESULTS

Two hundred thirty-six laboratories were located that were identified as likely to perform antiretroviral resistance testing. Of these, 165 (response rate, 69.9%) returned their survey. Included in the respondents were hospital laboratories (94, 57.0%), health department laboratories (29, 17.6%), independent laboratories (28, 17.0%) and an ‘other laboratories’ category (12, 7.3%), which included university medical research (six), federal research (two), clinical diagnostic company (one), research (one), and two that did not further specify the ‘other’. Most of the hospital laboratories were university based (40, 42.6%), privately owned (14, 14.9%), Department of Veterans Affairs (VA) (12, 12.8%) or community (8, 8.5%). Of the 29 health department laboratories, 15 (51.7%) were state, 7 (24.1%) were county, and 7 (24.1%) were ‘other’. Of the 28 independent laboratories, 16 (57.1%) considered themselves to be reference laboratories.

Of the 165 responding laboratories, only 23 (13.9%) performed HIV antiretroviral resistance testing. This testing was done predominantly by university hospital laboratories (11, 47.8%) or independent laboratories who further described themselves as reference laboratories (6, 26.1%). Of four testing laboratories that designated themselves in the ‘other’ category, three (75.0%) were further described as university medical school research laboratories, while one (25.0%) was self-described only as a research laboratory.

Among these 165 respondents, 63 (38.2%) did not perform HIV antiretroviral resistance testing, but did refer these tests to other laboratories, and the remaining 79 (47.9%) laboratories neither performed nor referred such tests.

The results discussed reflect the information obtained on those laboratories which did perform such testing. For example, of the 23 laboratories performing HIV-1 antiretroviral resistance testing, 22 participants completed the survey by responding to a majority, but not necessarily all, of the questions. One laboratory, however, responded only to the questions on laboratory type, i.e., research and indicated their personnel performed genotyping and phenotyping. Thus the results reported here reflect a varying response denominator for each question. All testing laboratories (n = 23) indicated that they used genotypic methods, while nine (39.1%) also used phenotypic methods. Most laboratories performing only genotyping were located in a hospital setting (9/14, 64.3%), while more laboratories which performed both genotyping and phenotyping were research (4/9, 44.4%) and independent laboratories (3/9, 33.3%).
Twenty-one laboratories had been performing the testing for a median of 19 months (range, 1 to 96 months). When laboratories were stratified for genotyping-only versus genotyping-plus-phenotyping methodology, the median number of months of testing experience was 12 and 31, respectively. The median number of tests for 22 laboratories was 41 per month (range, 5 to 2000 tests). When stratified for genotyping-only versus genotyping-plus-phenotyping, those laboratories performing genotyping-plus-phenotyping had a median monthly patient resistance test volume of 80 when compared with a median of 35 for the genotyping-only laboratories. Seventeen (77.3%) of the 22 laboratories performed this testing as a component of clinical trials (CT), 18 (81.8%) for laboratory research (LR), and only 12 (54.5%) for patient treatment (PT). Of the 12 laboratories testing PT specimens, seven laboratories or 50% (7/14) of the genotyping-only laboratories and five laboratories or 62.5% of the genotyping-plus-phenotyping laboratories tested for this purpose (PT). None of the genotyping-plus-phenotyping laboratories and only one of the genotyping-only laboratories performed testing solely for patient treatment.

Additionally, ten (45.4%) laboratories performed testing for all three reasons (PT, CT, LR) and five (22.7%) for two reasons (CT, LR). The first group (PT, CT, LR) had a median number of months of experience of 23 months, the second (CT, LR), 30 months.

**Specimen collection and treatment**

Guidelines for specimen stability and shipping varied considerably (Table 1). Twenty laboratories provided instructions for specimen collection, while two did not. Eighty percent of the laboratories that recommended plasma for genotyping to be collected in ethylenediaminetetraacetic acid (EDTA), while 50% of the laboratories indicated that plasma could be collected in citric acid, trisodium citrate, dextrose solution (ACD). When the data were stratified by purpose for testing, 11 of 12 laboratories doing PT and 15 of 17 laboratories doing CT indicated they provided instructions for specimen collection. All 14 of the CLIA-certified laboratories provided instructions for specimen collection, and six of the seven non-CLIA-certified laboratories also provided these instructions. Two laboratories performing testing for both CT and LR and one laboratory doing testing for only PT indicated that they did not provide information about specimen stability.

**Testing**

Seventeen testing laboratories responded to a question on what patient information they requested. Of these, nine (52.9%) requested and collected information about the patient’s HIV drug treatment history, 15 (88.2%) collected information about the patient’s viral load history or previous results, and 8 (47.1%) collected information about the patient’s previous CD4+ results.

Table 2 shows the responses for the purpose of testing (PT, CT, LR) and the type of patient information collected, i.e., HIV drug treatment history (DT), patient viral load history/previous results (VL),

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**Table 1.** Response to questions regarding laboratory practices for collection, handling, and treatment of specimens for antiretroviral resistance testing

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<th>ITEM</th>
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* Other = 1-cervical vaginal lavage; 1-frozen; 1-tissues for research, i.e., brain, lymph node, lung, etc.
† Other = 1-frozen, 2-plasma preparation tube (PPT)
‡ Other = 1-frozen, 1-plasma preparation tube (PPT)
§ Other = dry ice if >48 hrs; refrigerate if >24 hrs; ambient if <24 hrs.
and CD4+ history/previous results (CD4+). Five of nine (55.6%) laboratories testing for all three purposes collected information on DT; eight of these nine (88.9%) on VL, and four of nine (44.4%) on CD4+. One laboratory performing this test for both PT and CT and one laboratory performing this test for both CT and LR, did not collect information on any of the three: DT, VL, or CD4+. One laboratory performing testing for only PT did not collect information on DT. When stratified by genotyping-only versus genotyping-plus-phenotyping, all (7/7, 100%) of responding genotyping-plus-phenotyping laboratories collected information on VL, while 80% (8/10) of the genotyping-only laboratories collected this information. When considering collection of history on DT, 85.7% (6/7) of the genotyping-plus-phenotyping laboratories collected and 30% (3/10) of the genotyping-only laboratories collected such information. Only 30% (3/10) of the genotyping-only laboratories requested CD4+ information, while 71.4% (5/7) of the genotyping-plus-phenotyping requested this information.

Only ten (45.5%) of 22 laboratories tested patient specimens for viral load prior to, or concurrently with, antiretroviral resistance testing. When stratified by testing method, 8 (57.1%) of 14 of the genotyping-only laboratories and two (25.0%) of eight of the genotyping-plus-phenotyping laboratories tested for viral load prior to or concurrently with the antiretroviral resistance testing. Eighty percent indicated the reason for such testing was to determine if sufficient virus was present, 10% indicated it was a routine part of clinical care, and 10% indicated viral load would be performed if the patient had not had a viral load test within the last five months.

Respondent laboratories indicated which antiretroviral was used in their resistance testing procedures. All laboratories performing genotyping (n = 22) indicated resistance testing was performed for the nucleoside analogue reverse transcriptase inhibitors abacavir, didanosine (ddl), lamivudine (3TC), stavudine (d4T), zalcitabine (ddc), and zidovudine (AZT); for the non-nucleoside reverse transcriptase inhibitors efavirenz, delavirdine (DLV), and nevirapine (NVP); for the protease inhibitors indinavir, nelfinavir, ritonavir, and saquinavir. On the other hand, of the phenotyping laboratories (n = 8), resistance testing was performed by 75% of the laboratories for 3TC, AZT, indinavir, and nelfinavir; by 62.5% of the laboratories for ddl, d4T, ddc, NVP, ritonavir, and saquinavir; and by 50% of the laboratories for abacavir, efavirenz, and DLV. Investigational drugs, at the time of the survey, were amprenavir and adefovir for which 72.7% and 70.0%, respectively, were tested by the genotyping laboratories and 37.5% and 25.0%, respectively, were tested by phenotyping laboratories.

Ten laboratories indicated that they would be adding new drugs to their menu within the next six months. For those performing genotyping (15 responses), testing was offered per drug (6.7%), per panel (60.0%), or both (33.3%). For those performing phenotyping (seven responses), testing was offered per drug (57.1%), per panel (28.6%), or both (14.3%). The cost of genotypic testing for a single test ranged from $95 to $595 (median $398), and the cost for a panel ranged from $425 to $900 (median $450). Three laboratories, however, indicated they did not charge for a single test, and one laboratory did not charge for testing a panel of antiretrovirals. The cost for phenotypic testing for a single test (two responses) ranged from $250 to $400 (median $325), with an additional respondent performing the test at no charge, while for a panel (one response), the cost was $450.

Table 2. Type of patient history collected by purpose of testing (n = 17)

<table>
<thead>
<tr>
<th>PURPOSE†</th>
<th>INFORMATION COLLECTED*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DT</td>
</tr>
<tr>
<td>PT + CT + LR (n = 9)</td>
<td>5 (55.6%)</td>
</tr>
<tr>
<td>PT + CT (n = 0)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CT + LR (n = 3)</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>PT (n = 1)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>LR (n = 2)</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>CT (n = 1)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Other (n = 1)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
</tr>
</tbody>
</table>

* Information collected: DT = patient HIV drug treatment history; VL = patient viral load history/previous results; CD4+ = patient CD4+ previous results/history.
† Purpose of testing: PT = patient treatment, CT = clinical trials; LR = laboratory research; Other = epidemiologic surveillance
‡ Social security number (specified as ’optional’)
§ Patient adherence and study codes
Quality assurance
Of those performing genotyping testing, 20 (95.2%) out of 21 had a laboratory procedure manual with instructions for such testing, while only six (75.0%) out of eight of those performing phenotyping testing had such a manual. Of the 22 responding laboratories, 16 (72.7%) had criteria for rejecting samples. Table 3 displays the reasons for rejecting samples along with the number of laboratories ranking that criterion among the top three reasons for sample rejection. A specimen with too low a viral load, incorrect anticoagulant and specimen not at the appropriate shipping temperature were reasons most frequently mentioned for rejecting specimens. Respondents (n = 16) indicated that from zero to 200 specimens in a given month were rejected, with a median of three specimens.

Quality control practices
For those performing genotyping, quality control (QC) practices (n = 22), 20 (90.9%) included a negative control and 21 (95.5%) included a positive control. When laboratories were asked how frequently negative controls were run, responses included: daily; each run; with each set of seven samples; with new technologist training; weekly in every polymerase chain reaction (PCR) amplification; every run of 96; and with each batch of specimens. (It is unknown if the respondent considered a batch of samples to be a run.) Four laboratories used ‘other’ QC practices, indicated to be known proficiency test specimens; Pediatric AIDS Clinical Trial Group (PACTG); a patient sample; or samples run in parallel blinded with a second laboratory. Eleven (61.1%) of 18 reported that their controls were non-kit controls.

For those performing phenotyping QC procedures (n = 6), five (83.3%) ran a negative control and three laboratories specified the frequency with every run, every seven samples, or every run of 96. Six (100%) used a positive control with every run, every run of 96, every sample, or each batch of samples tested. One specified ‘other’ QC practice which was patient sample run on a monthly basis. Five responded that their controls were non-kit controls.

Sources of QC materials for genotyping included commercial (n = 11), in-house (n = 12) or another laboratory (n = 3). For phenotyping QC, sources of materials were primarily in-house (n = 7), with one laboratory using another laboratory as a source.

During a one-month period, laboratories (n = 21) estimated the number of patient specimen tests that needed to be repeated to range from zero to 200, with a median of five. The laboratory estimating 200 repeats per month averaged 2000 patient specimens tested per month. Laboratories (n = 20) reported the primary reasons for repeat testing to be: failure to amplify genetic material (n = 8); poor sequence quality (n = 3); inadequate amplification (n = 3); reproducibility (n = 2); low viral load (n = 1); physician request (n = 1); part of research protocol (n = 1); human error (n = 1).

Eighteen of the laboratories responded to a question about having an established written protocol for performing quality assurance (QA) activities. Of these 18, 13 (72.2%) indicated that they had such a protocol. Ten (76.9%) of 13 CLIA-certified laboratories and three (75%) of the non-CLIA-certified laboratories indicated they had such a written protocol. Over 77% of those performing testing for PT and over 84% performing testing for CT had such a protocol. VA hospital laboratories (n = 2) which, in one hospital, performed testing for PT and in the other, performed testing for LR, did not have a written protocol for QA activities.

Laboratories were asked if they would be willing to participate in a cost-free model performance evaluation program (MPEP) if it were available for HIV-1 antiretroviral resistance testing. Nineteen of 22 respondents (86.4%) indicated a willingness to participate in a laboratory QA program sponsored by CDC. Three
visor, regardless of laboratory type, while Table 4 indicates most testing personnel Personnel * Respondents were prompted to check all training that applied to their laboratory.

### Table 4. Type of training for testing personnel who perform antiretroviral resistance testing**

<table>
<thead>
<tr>
<th>TYPE</th>
<th>PERSONNEL n = 21</th>
<th>SUPERVISOR n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house training by supervisor</td>
<td>18 (85.7%)</td>
<td>——</td>
</tr>
<tr>
<td>Parallel/proficiency testing</td>
<td>14 (66.7%)</td>
<td>10 (50.0%)</td>
</tr>
<tr>
<td>In-house training by manufacturer</td>
<td>10 (47.6%)</td>
<td>11 (55.0%)</td>
</tr>
<tr>
<td>In-house training by peer</td>
<td>10 (47.6%)</td>
<td>4 (20.0%)</td>
</tr>
<tr>
<td>Test kit manufacturer training - off-site</td>
<td>9 (42.9%)</td>
<td>7 (35.0%)</td>
</tr>
<tr>
<td>Equipment manufacturer - off-site</td>
<td>5 (23.8%)</td>
<td>7 (35.0%)</td>
</tr>
<tr>
<td>Seminar/classroom course</td>
<td>5 (23.8%)</td>
<td>7 (35.0%)</td>
</tr>
<tr>
<td>No training</td>
<td>0 (0.0%)</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>Other, please specify:</td>
<td>0 (0.0%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>1-involved in developing the kit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-lead scientist who developed the assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-year of developing laboratory procedures</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Respondents were prompted to check all training that applied to their laboratory.
collected information on the patient’s vi-
ral load history (previous results) and only
45.5% performed this testing on each
specimen prior to, or concurrently with,
antiretroviral resistance testing.

Only 52.9% of the laboratories collected in-
formation about prior drug treatment, al-
though these data are also pertinent to inter-
preting test results and selecting antiretrovirals
to be used. Such testing represents informa-
tion on the predominant circulating variants
and may miss minor variants. The minor
variants can predominate if inappropriate
antiretrovirals are chosen.

Although the ordering of a laboratory test
is frequently left to the discretion of the
attending physician, it is the laboratory’s
role to provide advice to the physician re-
garding usefulness or appropriateness of
such testing. Such an approach tends to
increase the quality of health care service
to the patient. The responding laborato-
ries indicated that they provided inclu-
sive panels of antiretrovirals for consid-
eration by the physician. All (100%) of
the listed antiretrovirals were included for
those laboratories performing genotyping
(Table 3). Approximately 70% provided
test results for investigational drugs. This
practice allows the physician a complete
choice of drugs from which to select for
treatment and eliminates delays that
might occur due to the necessity for re-
ordering or expanded testing.

The physician, and consequently the pa-
tient, can best be served by the laboratory’s
provision of high-quality, informative, and
accurate test results. These study results
demonstrate great heterogeneity among
the kinds of laboratories that perform HIV-
1 antiretroviral resistance testing, the kind
of testing performed (genotyping-only or
genotyping-plus-phenotyping), and the
reason for testing, i.e., PT, CT, or LR. A
wide variety of protocols and procedures
appear to exist between laboratories.
Sources of training, length of training, and
purpose of testing were also inconsistent.
QA and QC practices varied from labora-
ory to laboratory regarding adherence to
existing requirement standards such as
CLIA ’88. In some settings, patient test-
ing, which requires the strictest applica-
tion of standards, may not be the focus of
the laboratory’s activities. A consensus of
what laboratories should be doing in the
arena of antiretroviral resistance testing
is clearly needed. Because of the heteroge-
eity among laboratories, it may be diffi-
cult to achieve compliance to existing stan-
dards and to create consensus in areas
where standards do not exist. On the other
hand, having few laboratories performing
this testing may facilitate the consensus
process. The benefit of successful consen-
sus building is that as more and more labo-
ratories undertake HIV-1 antiretroviral
testing, they will have guidelines and stan-
dards to follow.

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The opinions expressed are those of the
authors and do not necessarily reflect in-
stitutional policy.

Use of trade names and commercial sources
is for identification only and does not im-
ply endorsement by the U.S. Department
of Health and Human Services.

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Panel. Antiretroviral drug resistance testing in

Table 5. Types of questions asked by physicians of the respondent laboratories (n = 21) and number of laboratories ranking that question among the top three in frequency

<table>
<thead>
<tr>
<th>TYPE OF QUESTION</th>
<th>RESPONDENTS</th>
<th>TIMES RANKED</th>
<th>TIMES RANKED IN TOP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation of results</td>
<td>16</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Specimen collection, transportation, or</td>
<td>14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>temperature storage requirements</td>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cross-resistance</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Questions about accuracy</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Drug selection</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Other questions, please specify: price;</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>turnaround time; why no sequence from 10,000 copies/mL of virus</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drug levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No questions</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Objective: The study was undertaken to assess educators', practitioners', and managers' perceptions of the future job expectations of clinical laboratory scientists (CLSs) and their opinions on the skills that are expected of CLSs at entry-level and with experience.

Design: Survey participants were given a list of 44 competencies related to clinical laboratory science (CLS) practice and were asked whether they would expect a graduate of a respected CLS program to perform each competency in one of three educational categories: the first year of practice, with three to five years of experience but no additional education, or with three to five years of experience plus additional education. The competencies were subclassified into one of four major management functions: laboratory operations, human resource management, financial operations, or communications/consultation. Surveys also included eight Likert-type questions designed to assess the respondents' opinions on the future job expectations of CLS practitioners.

Participants: The sample for the survey included 280 directors of CLS educational programs, 600 managers randomly selected from the Clinical Laboratory Management Association (CLMA) membership, and 600 practitioners randomly selected from the American Society for Clinical Laboratory Science (ASCLS) membership.

Main Outcome Measures: The percent of respondents selecting each educational category was tabulated and each competency was assigned to one educational category based on the highest percent of respondents selecting that category. The means of the responses to the Likert-type questions were calculated for all respondents and for each group of respondents (educators, managers, and practitioners).

Results: Response rates of 58% (educators), 28% (practitioners), and 39% (managers) were obtained. Of the 44 competencies in the survey, four were expected at career-entry, 17 were expected of CLS graduates with work experience but no additional education, and 23 were expected of CLS graduates with experience plus additional education. Competencies expected in the first year of practice were primarily scientific and technical. With three to five years of practice and no additional education, the expectations for practitioners were primarily in laboratory operations and communications/consultation areas. The majority of the human resource management and financial operations competencies were expected with three to five years of practice and additional education. All participants agreed that CLS staff-level practitioners need more management and administrative skills and that, in the future, CLS practitioners will spend less time performing laboratory tests and more time solving problems. CLS managers were more positive than CLS educators in response to statements asserting that CLT practitioners and non-certified personnel will have an increased role in the laboratory in the future.

Conclusion: This study suggests that extensive laboratory operations and communication skills are expected of CLS graduates without any additional education beyond their CLS programs. CLS educators should adequately address those areas in the curriculum. Competence in other non-technical skills may not be expected without the benefit of post-baccalaureate education and in these areas, CLS programs can provide a foundation for future learning.

Abbreviations: ASCLS = American Society for Clinical Laboratory Science; CE = continuing education; CLS = clinical laboratory science; CLSs = clinical laboratory scientists; CLT = clinical laboratory technician; MT = medical technologist; NAACLS = National Accrediting Agency for Clinical Laboratory Sciences.

Index Terms: Clinical laboratory science; curriculum; education; laboratory personnel; medical technologist.
As clinical laboratory science (CLS) educators design and revise curricula, they must identify the competencies that graduates will need for professional practice. The competencies addressed in CLS curricula should include those needed for career-entry and those that will enable graduates to assume leadership roles in laboratory science, education, and management. Career-entry expectations of clinical laboratory scientists (CLSs) have been well documented in the National Credentialing Agency for Laboratory Personnel (NCA) Job Analysis studies. These national surveys of educators, practitioners, and employers identified the competencies that are important in the first year of practice. In both the 1993–94 and 1998–99 job analyses, the majority of competencies expected of CLSs at entry-level were in scientific and technical areas. These scientific and technical skills ranged from very simple competencies such as routine urinalysis to highly complex techniques such as molecular methods for identifying microorganisms.

The competencies needed for CLS practitioners beyond entry-level are not as well defined. It is clear that the expectations and responsibilities of CLSs increase with work experience. A recent study of the job responsibilities of laboratory professionals found that after five years of practice, CLSs were performing more advanced technical skills and more management tasks. Gardner and Estry studied CLS practitioners over the ten year period between 1983 and 1993 and found that job responsibilities shifted toward higher-level technical activities and management activities such as marketing services, budget control, quality assurance, and documentation. Evidence of increasing job responsibilities for laboratory professionals after career-entry also comes from the NCA job analyses. The surveys used in the 1998-99 NCA job analysis contained over 80 tasks in the area of management, safety, quality assurance, and consultation. Respondents indicated that most of these tasks were not performed by entry-level CLS practitioners; however, they were being performed in the laboratories. It is likely that laboratory professionals with more experience and possibly more education are performing these higher-level tasks.

Because entry-level CLSs are expected to have extensive technical skills, CLS educators devote most of their curricula to the principles, performance, and interpretation of laboratory testing. To meet accreditation standards and to prepare students for future job responsibilities, CLS educators have also included management and education competencies in the curriculum. However, the amount of time devoted to, and the content covered in these non-technical areas has been limited by the need to cover so many scientific and technical aspects of laboratory practice.

In 2000, the National Accrediting Agency for Clinical Laboratory Science (NAACLS) held a conference to discuss the future roles of clinical laboratory technicians (CLTs) and CLSs and to address changes in curricula needed to prepare graduates for these roles. Participants at the NAACLS conference described the CLT of the future as the practitioner who will perform the majority of bench tests. The description of the future CLS practitioner included responsibility for in-depth analysis of data, esoteric testing, research and development, management, and consulting. The results of these discussions on the future of clinical laboratory practice are reflected in the recently adopted NAACLS Standards for Accredited CLS/MT programs. According to the NAACLS Standards, accredited CLS curricula should include principles of critical pathways, clinical decision making, performance improvement, and dynamics of healthcare delivery systems, human resource management, and financial management.

To design curricula, CLS educators must be both practical and prophetic. They must ensure that students are prepared for their first jobs, which will most likely require scientific and technical expertise, and they must provide students with the knowledge, skills, and attitudes they will need to advance in their professional responsibilities. Because education does not stop after graduation, CLS educators can assume that some advanced knowledge will be added after baccalaureate education either through continuing education (CE) or formal coursework. Some responsibilities, however, may be expected of graduates without the benefit of any additional CE and, in these situations, practitioners will rely on their undergraduate CLS education for preparation.

This study was undertaken to provide additional information on the job expectations of CLS practitioners at entry-level and with experience in order to inform curriculum decisions. Specifically the study asked:

1. What are educators', managers', and practitioners' perceptions of the educational preparation and future job expectations of CLSs?
2. What are the implications of these perceptions for CLS curricula?
3. What skills are expected of CLSs in three educational categories: at entry level, in the first three to five years of practice with no additional education, and in the first three to five years of practice with additional education?
4. What are the curricular implications of the skills expected of CLSs in each educational category?

METHOD

This study was a component of a national study assessing laboratory practitioners', educators', and managers' views on educational preparation and job expectations of CLSs. A complete description of the method is provided in Beck and Doig, 2002. With the assistance of an advisory board consisting of educators, managers, and practitioners, the authors developed a survey to assess the competencies expected of CLSs at entry-level and after work experience. A CLS was defined as someone who had obtained a baccalaureate degree, attained national certification, and used independent judgment to provide laboratory information and services.

Two hundred eighty directors of NAACLS accredited CLS programs were selected to serve as the sample of CLS educators. Six hundred managers were randomly selected from the Clinical Labo-
ratory Management Association (CLMA) mailing list and 600 practitioners were randomly selected from the American Society for Clinical Laboratory Science (ASCLS) mailing list.

Surveys included demographic questions on geographic region, work setting, annual volume of tests, primary job function, highest degree, and years of paid experience. Surveys also included eight Likert-type questions designed to assess the respondents’ opinions on the current and future job expectations of CLS practitioners (Table 1). Respondents were asked to read each statement and indicate their opinion using a scale on which 1 = strongly disagree and 5 = strongly agree. Practitioners were asked how many hours of continuing education they attended each year, whether or not continuing education was a requirement for their current job, the means they used to obtain continuing education, and whether or not they had taken graduate-level courses.

Respondents were given a list of 44 competencies related to CLS practice and were asked whether they would expect a graduate of a respected CLS program with excellent grades, good recommendations, and coursework in laboratory management, e.g., supervision, budgeting, quality management, and marketing to perform each competency in the first year of practice, with three to five years of experience but no additional education, or with three to five years of experience plus additional education, e.g., continuing education (CE) or formal courses. Respondents could also select none of these options. The list of competencies was developed using the NCA job analysis task lists, NAACLS CLS Standards, and the expertise of the advisory panel. Because previous studies had identified the scientific and technical competencies expected of CLSs, this list of competencies emphasized non-technical aspects of clinical laboratory practice.

The surveys and cover letters, including the definitions of terms, were tested in a pilot study using a sample of educators, managers, and practitioners from across the U.S. The surveys and definitions were revised based on comments from participants in the pilot study. Surveys were sent to the CLS educators, managers, and practitioners in March 2000. Surveys received within six weeks were included in the data analysis.

DATA ANALYSIS
SPSS 9.0 for Windows was used to analyze the data collected in this study. The means of the responses to the Likert-type questions were calculated for all respondents and for each group of respondents (educators, managers, and practitioners). Participants’ responses to the Likert-type survey questions were classified using the following criteria: disagree = mean score of 2.5 or less; undecided = 2.5 and 3.5; agree = 3.5; significance (p < 0.01)

Table 1. Mean responses of all respondents (ALL), educators (EDU), managers (MAN), and practitioners (PRAC) to statements on CLS educational preparation and job expectations

<table>
<thead>
<tr>
<th>STATEMENT</th>
<th>ALL</th>
<th>EDU</th>
<th>MAN</th>
<th>PRAC</th>
<th>SIGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In today’s clinical laboratory, CLS/MT level staff members need more management and administration skills.</td>
<td>3.70</td>
<td>3.69</td>
<td>3.80</td>
<td>3.58</td>
<td>.14</td>
</tr>
<tr>
<td>2. CLS/MT practitioners of the future will spend more time solving problems and less time performing laboratory tests.</td>
<td>3.67</td>
<td>3.76</td>
<td>3.71</td>
<td>3.52</td>
<td>.05</td>
</tr>
<tr>
<td>3. In today’s clinical laboratories, i.e., hospitals, commercial laboratories, and POLs, CLS/MT practitioners produce most of the billable test results.</td>
<td>3.61</td>
<td>3.78</td>
<td>3.46</td>
<td>3.64</td>
<td>.01</td>
</tr>
<tr>
<td>4. Non-certified personnel will constitute a significant proportion of the laboratory staff in the future.</td>
<td>2.84</td>
<td>2.42</td>
<td>3.07</td>
<td>2.93</td>
<td>.00</td>
</tr>
<tr>
<td>5. Baccalaureate-level CLS/MT programs are adequately preparing students for the future clinical laboratory environment.</td>
<td>3.54</td>
<td>3.70</td>
<td>3.37</td>
<td>3.63</td>
<td>.00</td>
</tr>
<tr>
<td>6. In the future, there will be a need for more CLT/MLT level practitioners and fewer CLS/MT level practitioners.</td>
<td>3.31</td>
<td>3.06</td>
<td>3.48</td>
<td>3.30</td>
<td>.00</td>
</tr>
<tr>
<td>7. Baccalaureate degree CLS/MT programs should focus on the sciences underlying laboratory testing, not on management and education.</td>
<td>3.03</td>
<td>3.20</td>
<td>2.83</td>
<td>3.16</td>
<td>.00</td>
</tr>
<tr>
<td>8. In the future, associate-degree CLT/MLT practitioners will be doing the majority of laboratory testing.</td>
<td>3.42</td>
<td>3.17</td>
<td>3.62</td>
<td>3.39</td>
<td>.00</td>
</tr>
</tbody>
</table>

Disagree = ≤2.5, Undecided = >2.5 and ≤3.5, Agree = ≥3.5; SIGN = significance (p <0.01)
undecided = mean score greater than 2.5 and less than 3.5; and agree = mean score of 3.5 or higher. Analysis of variance was used to assess differences in responses among groups. The level of significance was set at a p value of less than 0.01 and significant differences were analyzed using the LSD (least significant difference) and the Tukey’s honestly significant difference tests.

The percent of respondents selecting each educational category (in the first year of practice, with three to five years of experience but no additional education, with three to five years of experience plus additional education, or none of these) was tabulated. Each competency was assigned to one educational category based on the highest percent of respondents selecting that category. The authors independently sub-classified the competencies in one of four major management functions: laboratory operations, human resource management, financial operations, or communications/consultation. Differences in sub-classifications were discussed and a final assignment was determined by consensus.

RESULTS
Response rate
Usable surveys were received from 163 educators (58%), 231 managers (39%), and 166 practitioners (28%) for a total of 560 (38%) respondents.

Demographic information
A complete description of the demographic information from this study is provided in Beck and Doig, 2002. The respondents in each group came from all geographic regions and institutions of all sizes. The majority of managers (74.9%) and practitioners (63.1%) worked in hospitals or medical centers and most educators indicated that they worked either in a hospital/medical center (46%) or an educational program (41.1%). Eighty-eight percent of NAACLS program directors indicated that their primary job function was educator and over 95% of the CLMA members listed their job function as supervisor, administrator, or director. Most of the respondents in the practitioner survey (55.4%) listed CLS or medical technologist (MT) as their primary job function. The majority of the managers (60.2%) and practitioners (68.5%) indicated that a baccalaureate degree was their highest degree. Respondents in the educator group had the highest percent of master’s degrees (54.6%) and doctorates (28.8%). The educator group was also the oldest group of respondents with 79.8% reporting that they had over 20 years of paid experience. The practitioners were younger than the educator and manager groups, with 50% of practitioners indicating that they had fewer than 20 years of experience.

Practitioners’ continuing education
Over half of the practitioners (56.4%) reported that they attended 11 or more hours of CE each year. Nineteen percent of the respondents attended between six and ten hours of CE, 16.8% attended between three and five hours of CE, and 7.8% reported attending between zero and two hours of CE each year. One half of the practitioners stated that CE was a requirement for their current job.

Practitioners obtained CE in a variety of ways with external CE programs cited by the highest percent of respondents (88%). The other means of obtaining CE included in-house CE programs (83%), journal articles (69%), selected graduate level courses (25%), graduate level degree programs (21%), courses on the Internet (14%), and mail or audioconferences (2%).

Ninety (53.5%) of the practitioners indicated that they had taken graduate level courses. They described the emphasis of these courses as scientific topics (38%), management/business (31%), education/training (18%), a combination of scientific, management, and education courses (11%), and computers (2%).

Opinions on current and future CLS practice
Responses of educators, managers, and practitioners to eight questions related to CLS educational preparation and job expectations are shown in Table 1. All groups agreed that CLS staff-level practitioners need more management and administrative skills and that, in the future, CLS practitioners will spend less time performing laboratory tests and more time solving problems (statements 1 and 2). Managers were undecided while educators and practitioners agreed with the statement that CLSs produce the most billable results in today’s clinical laboratory (statement 3); however, the differences in responses to statement 3 among these three groups were not significant.

Significant differences in the responses of educators and managers were detected in the analysis of statements 4, 5, 6, 7, and 8. Educators were more negative (more in disagreement) than managers when responding to statements suggesting that there will be an increased role for non-certified personnel (statement 4) and for CLT practitioners (statements 6 and 8) in the future. Educators were more positive (more in agreement) than managers in response to the statements asserting that BS programs should focus on science rather than management (statement 7).

Practitioners’ responses for all eight statements were not significantly different from managers’ responses. Practitioners differed from educators, however, in their responses to statement 4 which suggested that there will be more non-certified personnel in the future. Practitioners’ responses were more positive (more in agreement) than educators in response to this statement.

Competencies expected of CLSs
In Table 2, the percent of all respondents (educators, managers, and practitioners combined) who classified a competency into each educational category is listed. Competencies are listed below in the educational category selected by the highest percent of respondents. The competencies in each educational category are further
Table 2. Percent of all respondents (n = 560) classifying competencies in educational categories

<table>
<thead>
<tr>
<th>COMPETENCY</th>
<th>EDUCATIONAL CATEGORY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First year</td>
</tr>
<tr>
<td>1. Perform routine testing in all areas of the clinical laboratory.</td>
<td>94.5</td>
</tr>
<tr>
<td>2. Perform advanced testing, e.g., flow cytometry, DNA analysis.</td>
<td>21.4</td>
</tr>
<tr>
<td>3. Explain the basic analytical principles involved in laboratory procedures.</td>
<td>86.6</td>
</tr>
<tr>
<td>4. Resolve problems encountered in performing routine laboratory tests.</td>
<td>67.3</td>
</tr>
<tr>
<td>5. Establish protocols for acceptance or rejection of assay data based on quality control results.</td>
<td>51.0</td>
</tr>
<tr>
<td>6. Design protocols for monitoring or maintaining instruments and equipment.</td>
<td>22.9</td>
</tr>
<tr>
<td>7. Participate in decisions regarding laboratory instrumentation or equipment purchases.</td>
<td>11.1</td>
</tr>
<tr>
<td>8. Participate on laboratory and hospital committees; safety, transfusion, utilization review, etc.</td>
<td>13.4</td>
</tr>
<tr>
<td>9. Write or edit job descriptions.</td>
<td>7.7</td>
</tr>
<tr>
<td>10. Recruit and hire staff in compliance with current labor laws or regulatory standards.</td>
<td>3.2</td>
</tr>
<tr>
<td>11. Coach staff members to improve job performance.</td>
<td>5.0</td>
</tr>
<tr>
<td>12. Use principles of leadership and delegation to supervise and motivate staff.</td>
<td>3.2</td>
</tr>
<tr>
<td>13. Prepare staff schedules.</td>
<td>17.5</td>
</tr>
<tr>
<td>14. Conduct and evaluate clinical instruction and continuing education for laboratory personnel.</td>
<td>6.2</td>
</tr>
<tr>
<td>15. Develop and implement programs to document employee competency in the laboratory.</td>
<td>3.3</td>
</tr>
<tr>
<td>16. Develop programs that comply with federal regulations, e.g., CLIA, and voluntary accrediting requirements, e.g., CAP, JCAHO.</td>
<td>3.2</td>
</tr>
<tr>
<td>17. Interpret regulations and write procedures for safety, e.g., blood borne pathogens, chemicals, fire, and sharps.</td>
<td>8.7</td>
</tr>
<tr>
<td>18. Develop or revise a disaster plan.</td>
<td>5.3</td>
</tr>
<tr>
<td>19. Write laboratory procedures and manuals.</td>
<td>17.6</td>
</tr>
<tr>
<td>20. Establish guidelines for confidential handling of laboratory results and personnel information.</td>
<td>14.3</td>
</tr>
<tr>
<td>21. Monitor and troubleshoot daily operations of a computer system.</td>
<td>21.0</td>
</tr>
<tr>
<td>22. Design, implement, and evaluate QA and CQI (continuous quality improvement) procedures.</td>
<td>6.4</td>
</tr>
<tr>
<td>23. Assess current methods and evaluate the need to adopt new methods.</td>
<td>8.0</td>
</tr>
<tr>
<td>24. Coordinate proficiency testing.</td>
<td>18.9</td>
</tr>
<tr>
<td>25. Perform turn around time studies, i.e., from patient to laboratory to reported result.</td>
<td>31.0</td>
</tr>
<tr>
<td>26. Coordinate laboratory services with other departments to improve patient care.</td>
<td>11.8</td>
</tr>
<tr>
<td>27. Analyze laboratory and patient data to improve laboratory test utilization and services.</td>
<td>9.3</td>
</tr>
<tr>
<td>28. Evaluate and select capital equipment purchases including laboratory information systems.</td>
<td>2.0</td>
</tr>
<tr>
<td>29. Consult with other healthcare providers regarding analytical aspects of laboratory services.</td>
<td>13.1</td>
</tr>
<tr>
<td>30. Develop and implement test strategies, e.g., test sequencing and clinical pathways for use in practice guidelines.</td>
<td>3.2</td>
</tr>
<tr>
<td>31. Develop a business plan for laboratory operations.</td>
<td>0.4</td>
</tr>
<tr>
<td>32. Develop public relations programs for client services.</td>
<td>3.0</td>
</tr>
<tr>
<td>33. Market new laboratory services.</td>
<td>3.4</td>
</tr>
<tr>
<td>34. Develop and implement outreach programs for laboratory tests and services.</td>
<td>1.4</td>
</tr>
<tr>
<td>35. Prepare a laboratory or departmental budget.</td>
<td>2.7</td>
</tr>
<tr>
<td>36. Develop and implement a compliance plan for reimbursement and medical necessity requirements.</td>
<td>0.9</td>
</tr>
<tr>
<td>37. Negotiate contracts for laboratory services.</td>
<td>0.4</td>
</tr>
<tr>
<td>38. Evaluate patients’ laboratory results and determine the need for additional tests.</td>
<td>32.9</td>
</tr>
<tr>
<td>39. Perform method evaluation studies to adopt new methods.</td>
<td>23.6</td>
</tr>
<tr>
<td>40. Monitor current test costs.</td>
<td>19.3</td>
</tr>
<tr>
<td>41. Evaluate the need and decide whether to outsource laboratory tests or services.</td>
<td>5.5</td>
</tr>
<tr>
<td>42. Consult with other healthcare providers regarding the significance and value of laboratory results.</td>
<td>14.2</td>
</tr>
<tr>
<td>43. Address ethical questions related to laboratory testing and services.</td>
<td>24.4</td>
</tr>
<tr>
<td>44. Analyze research data and apply results to current laboratory practice.</td>
<td>12.5</td>
</tr>
</tbody>
</table>
classified into one of four management functions (laboratory operations, human resource management, financial operations, and communications/consultation).

First year of practice:
Laboratory operations:
• Perform routine testing in all areas of the clinical laboratory. (94.5%)
• Explain the basic analytical principles involved in laboratory procedures. (86.6%)
• Resolve problems encountered in performing routine laboratory tests. (67.3%)
• Establish protocols for acceptance or rejection of assay data based on quality control results. (51.0%)

Three to five years of experience but no additional education:
Laboratory operations:
• Coordinate proficiency testing. (60.5%)
• Perform turn around time studies, i.e., from patient to laboratory to reported result. (54.2%)
• Write laboratory procedures and manuals. (52.8%)
• Assess current methods and evaluate the need to adopt new methods. (50.4%)
• Design protocols for monitoring/maintaining instruments and equipment. (48.6%)
• Establish guidelines for confidential handling of laboratory results and personnel information. (47.3%)
• Analyze laboratory and patient data to improve laboratory test utilization and services. (47.1%)
• Perform method evaluation studies to adopt new methods. (45.2%)
• Evaluate patients’ laboratory results and determine the need for additional tests. (33.8%)
• Address ethical questions related to laboratory testing and services. (32.8%)

Human resource management:
• Prepare staff schedules. (65.9%)

Financial operations:
• Participate in decisions regarding laboratory instrumentation or equipment purchases. (58.0%)
• Monitor current test costs. (48.8%)

Communications/consultation:
• Participate on laboratory and hospital committees (safety, transfusion, utilization review, etc.). (61.0%)
• Coordinate laboratory services with other departments to improve patient care. (60.5%)
• Consult with other healthcare providers regarding analytical aspects of laboratory services. (44.2%)
• Consult with other healthcare providers regarding the significance and predictive value of laboratory results. (38.9%)

Three to five years of experience plus additional education, e.g., CE or formal courses:
Laboratory operations:
• Develop and implement a compliance plan for reimbursement and medical necessity requirements. (73.9%)
• Develop programs that comply with federal regulations, e.g., CLIA and voluntary accrediting requirements, e.g., CAP, JCAHO. (67.9%)
• Develop and implement outreach programs for laboratory tests and services. (59.5%)
• Interpret regulations and write procedures for safety, e.g., blood borne pathogens, chemicals, fire, and sharps. (53.7%)
• Design, implement, and evaluate QA and CQI (continuous quality improvement) procedures. (53.5%)
• Develop or revise a disaster plan. (48.8%)
• Develop and implement test strategies, e.g., test sequencing and clinical pathways for use in practice guidelines. (48.2%)
• Analyze research data and apply results to current laboratory practice. (43.7%)
• Perform advanced testing, e.g., flow cytometry, DNA analysis. (41.5%)
• Monitor and troubleshoot daily operations of a computer system. (37.8%)

Human resource management:
• Recruit and hire staff in compliance with current labor laws or regulatory standards. (67.1%)
• Use principles of leadership and delegation to supervise and motivate staff. (60.5%)
• Conduct and evaluate clinical instruction and continuing education for laboratory personnel. (57.6%)
• Develop and implement programs to document employee competency in the laboratory. (55.7%)
• Coach staff members to improve job performance. (48.5%)
• Write or edit job descriptions. (44.7%)

Financial operations:
• Develop a business plan for laboratory operations. (73.5%)
• Negotiate contracts for laboratory services. (67.9%)
• Prepare a laboratory or departmental budget. (61.5%)
• Evaluate and select capital equipment purchases including laboratory information systems. (54.2%)
• Evaluate the need for and decide whether to outsource laboratory tests or services. (42.3%)

Communications/consultation:
• Develop public relations programs for client services. (58.6%)
• Market new laboratory services. (55.8%)

Of the 44 competencies in the survey, four were expected at career-entry, all sub-classified in laboratory operations. Seventeen competencies were expected of CLS graduates with work experience but no additional education. Ten of these competencies were
in laboratory operations, one was in human resources, two were in financial management, and four were in the communications/consultation sub-classification. Twenty-three of the competencies on this survey were expected of CLS graduates with experience plus additional education. In this educational category, ten competencies were sub-classified as laboratory operations, six were in human resource management, five were in financial management, and two were in communications/consultation.

DISCUSSION

There was general agreement among educators, practitioners, and managers that CLSs are currently doing the majority of laboratory testing and will be doing less testing and more problem solving in the future. There were differences of opinion among the respondent groups, however, when they were asked about an increased role for CLTs and non-certified personnel. Educators had the strongest negative response to the suggestion that there will be more non-certified personnel in the laboratory of the future. Educators were also significantly more negative than managers when asked if CLTs would be performing more testing in the future. If CLTs are doing less testing, it is not clear who educators see as the major testing personnel in the future. CLS educators may be reluctant to envision a future in which CLTs or non-certified personnel replace CLS practitioners. Alternatively, CLS educators may think that changes in technology alone could result in CLTs spending less time performing laboratory tests and more time solving problems.

All groups agreed that CLSs need more management and administrative skills and that the problem solving roles of CLSs will increase in the future. These findings are consistent with the description of future CLS practitioners generated at the NAACLS conference and they validate the need for non-technical skills, such as management and administration in the CLS curriculum. It is interesting to note, however, that the educators and managers in this study differed significantly in their responses to two statements addressing how CLS programs are preparing students for their future roles. Educators agreed with the statement that CLS programs were adequately preparing students for the future while managers were undecided in response to this question. Educators also felt more strongly than managers that the CLS curriculum should emphasize the sciences underlying laboratory testing rather than management and education. The fact that managers and educators in this study differed in their views of the adequacy of the current CLS education and the appropriate content in BS curriculum may be cause for concern. This highlights the need for ongoing communication between these two groups of laboratory professionals to ensure that managers understand the scope and limits of the CLS curriculum and educators understand the real job expectations of CLS practitioners.

The respondents’ classification of competencies in each of three educational categories provides useful descriptions of CLS practitioners and their job responsibilities. Only a few of the competencies on the survey were classified by the respondents as expected at entry-level. This was not surprising given that the survey emphasized non-technical competencies that had been described as beyond entry-level in previous studies. All of the competencies classified as expected at career-entry were in the area of laboratory operations and included performing tests, resolving testing problems, explaining results, and using quality control data. The emphasis on the scientific and technical skills in the first year of practice is consistent with the entry-level expectations identified in NCA job analysis studies. The low number of management skills included in this educational category is also consistent with previous studies addressing management skills expected of entry-level practitioners.

Competencies classified in the second educational category, expected within three to five years of practice without additional CE or coursework, included those sub-classified in the laboratory operations, human resource management, financial operations, and communications/consultation areas. Laboratory operations competencies went beyond performing tests to encompass an understanding of the total testing process. They included proficiency testing, turn around time studies, writing procedures, and method evaluation. Only one competency in human resource management, preparing staff schedules, was expected at this level. Financial management expectations were also minimal and included participating in purchasing decisions and monitoring test costs. The communications/consultations expectations of a practitioner with several years of experience, however, were significant. The practitioner was expected to effectively represent the laboratory in committees, coordinate laboratory services with other departments, and explain the analytical aspects, significance, and predictive value of laboratory tests to others. The picture that emerges of the CLS practitioner with three to five years experience is one who is competent in laboratory testing, involved in all aspects of the testing process, and able to consult with other healthcare professionals on issues related to laboratory testing.

The competencies that are classified in the third educational category, expected with three to five years of practice plus additional education, were sophisticated and broad. The laboratory operations competencies included specialized testing, e.g., flow cytometry and DNA analysis as well as competencies that required an understanding of the healthcare system and regulatory agencies that affect laboratory services. Most of the competencies in human resource management and financial management were found in this third category. Communication/consultation competencies involved articulating the laboratory’s services to others outside of the individual’s institution.

The respondents’ classification of competencies in each of three educational categories provides a guide for CLS curriculum planning. Competencies classified in the first and second educational categories represent the knowledge, skills, or attitudes that must
be addressed in the CLS curriculum because they will not necessarily be addressed by additional education after graduation. The emphasis on technical skills in the first year of practice and the emphasis on communication skills in three to five years of practice without additional education underscore the importance of the scientific and technical content in the CLS curriculum. Laboratory practitioners will rely on their undergraduate education to prepare them for performing all aspects of laboratory testing and for explaining that information and the role of the laboratory to others. The competencies that are classified in the third educational category, expected with three to five years of practice plus additional education, do not need to be completely addressed in the CLS curriculum, although the CLS curriculum should lay a foundation for future education in these areas.

The results of this study can be used by educators in conjunction with the NAACLS Standards to make decisions about curriculum content. The NAACLS Standards are intended to provide broad guidelines for CLS curricula. The results of this study provide more specific descriptions of the competencies that should be included in the CLS curriculum. For example, the recently adopted NAACLS Standards describe the entry-level CLS as one who has basic knowledge in "communications to enable consultative interactions with members of the healthcare team, external relations, customer service and patient education". CLS curricula are required to include "principles of interpersonal and interdisciplinary communication and team-building skills". The study included descriptions of six competencies related to communication and consultations, most of which were categorized as competencies expected of graduates within three to five years of practice without additional education. This provides educators with more concrete information about the communication/consultation skills needed and confirms the importance of addressing these skills in the CLS curriculum.

Educators can also use the results of this study to make decisions about the amount of emphasis needed in a particular area. For example, the proposed NAACLS Standards state that the CLS curriculum should include concepts and principles of "human resource management to include position description, performance evaluation, utilization of personnel, and analysis of workflow and staffing patterns". In this study, only one out of seven human resource management competencies, preparing staff schedules, was expected without additional education. The CLS curriculum should, therefore, give students the skills they need to prepare staff schedules; however, for other human resource management competencies, only a foundation for future learning is needed.

The results of this study emphasize the importance of both the BS curriculum and CE in the professional development of CLSs. The 21 competencies included in the first two educational categories must be addressed in the BS curriculum and the 23 competencies that were in the third educational category (experience plus additional education) provide an outline for continuing education programs or for a master's degree curriculum. The number of CLSs who are regularly participating in CE activities indicates that practitioners need additional education to maintain competence and advance in their jobs. Most practitioners were obtaining CE through traditional external and internal presentations. At the time of this survey, Internet courses were not used extensively; however, that may change in the future and be reflected in studies of this type.

LIMITATIONS
This survey was limited by the date of sampling and by length. The survey reflects the views of educators, practitioners, and managers at one point in time. It should be repeated in three to five years to detect changes in opinions and expectations. The comprehensiveness of the survey was limited by the need to keep the length of the survey reasonable. The response rates for this survey were considered good for a national, unsolicited survey indicating that the survey length was not overwhelming.

It must be noted that the classification of competencies into educational categories was based on the percent of respondents selecting a category and, in some cases, the responses were split across several categories making the classification less definite. The first competency on the survey is a clear-cut result. Approximately 95% of the respondents classified "perform routine testing in all areas of the clinical laboratory", as expected in the first year of practice. A competency that is less clearly classified is illustrated in Figure 1. Competency number 38, "Evaluate patient's laboratory results and determine the need for additional tests", was classified as expected in the first three to five years of practice based on the views of 34% of the respondents. However, 33% of the respondents felt that this competency could be expected in the first year of practice and 21% indicated it would be expected after obtaining experience and CE. In cases in which the responses were split between several categories, educators can use the percentages in all categories to decide how much emphasis the competency should receive in the curriculum. For the example in Figure 1, most respondents placed this competency in an educational category that relied on the BS curriculum for preparation (either first year of practice or with experience but no CE). Educators therefore would want to address this competency in the CLS curriculum.

SUMMARY
The results of this study shed light on the current discussions concerning the appropriate curriculum for CLS programs. While there is general agreement that CLS programs must adapt to meet the needs of the future, and that adaptation includes adding more non-technical skills, it is not clear what those skills are. This study suggests that laboratory operations skills that require an understanding of the total testing process and communication skills are expected of CLS graduates without any additional education beyond their CLS programs. CLS educators should adequately address those areas in the curriculum. Competence in other non-
technical skills may not be expected without the benefit of post-baccalaureate education and in these areas, CLS programs can provide a foundation for future learning. This study highlights the importance of entry-level education in CLS programs and the need for on-going professional education after graduation.

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REFERENCES


2002 Reviewer Thank You

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Newborn Screening: An Overview

EILEEN CARREIRO-LEWANDOWSKI

The ethical considerations and the criteria for inclusion of a test to a newborn screening program have remained constant since testing began in the 1960s. Does the test identify a treatable disorder with significant incidence to pose a public health risk and warrant testing all babies in that state or territory? Technological advances in testing, particularly with the improvement of tandem mass spectrometry techniques and the advent of DNA testing for the specific gene mutations, have expanded our understanding of many inherited metabolic diseases. These mostly autosomal recessive disorders went under-diagnosed by the medical community for many years. This was partly due to the notion that the incidence of inherited metabolic diseases was quite rare and that many so-called birth defects, or unexplained infant deaths, were not associated with any known metabolic disorders.

Public health departments, as part of their newborn health programs, offer some newborn screening to all infants born within their jurisdiction. Two tests, those for phenylketonuria (PKU) and congenital hypothyroidism are universally mandated (51/51 jurisdictions). The next highest frequency tests are for galactosemia and sickle cell disease (50/51), with up to thirty tests available in some states. However, the authority as to which tests are included resides with the local state government, either as a matter of law or as a matter for the public health department. As these matters become more complex, many public health officials and pediatric healthcare practitioners urge the Federal government to become involved and develop national guidelines in an effort to streamline the process and decrease the existing inconsistencies between states.

For many laboratorians, the collection of newborn screening blood spot samples is the extent of their involvement in newborn screening programs. The many facets of these programs, the status of newborn screening in the United States, and the incidence and description of selected inherited disorders are explored.

ABBREVIATIONS: BIO = biotinidase deficiency; CAH = congenital adrenal hyperplasia; CF = cystic fibrosis; CH = congenital hypothyroidism; FOD = fatty acid oxidation disorders which include MCAD (medium chain acyl-CoA dehydrogenase deficiency); GAL = galactosemia; HCY = homocystinuria; MS/MS = tandem mass spectrometry; MSUD = maple syrup urine disease; OAA = other amino acidemias including tyrosinemia types I and II; OAD = organic acidemias; PKU = phenylalaninemia; SCD = sickle cell disease.

INDEX TERMS: newborn diseases; newborn testing.

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Focus Continuing Education Credit: see pages 245 to 247 for learning objectives and application form.

LEARNING OBJECTIVES

At the end of the article, the learner will be able to:
1. Identify the ethical issues surrounding newborn screening.
2. Discuss the impact of nationally adopted standards on newborn screening.
3. Describe the criteria used for incorporation of a test in a newborn screening program.
4. Discuss the impact of technological advances on newborn screening.
5. Explain the principle of tandem-mass spectrometry.
6. Identify the pre- and post-analytical variables related to newborn screen testing.
7. Define the deficiency and discuss the physiologic implications associated with the inherited metabolic diseases presented.
8. Discuss the relative incidence of the 12 inherited disorders described.
9. Compare voluntary and mandatory screening.
10. List the factors that historically impeded early diagnosis of inherited metabolic diseases.
11. List the two disorders screened by all U.S. states.
12. List the 15 states that offer testing (at least MCAD) for fatty acid oxidation disorders.

The idea of newborn screening began with testing for an inherited disorder, phenylketonuria, in 1962. Advances in medical research and analytical techniques have led to the discovery and ability to test for many inherited metabolic diseases and other birth disorders. The more recent (1990s) development of newborn screening methods using tandem mass spectrometry (MS/MS) allows for a wide array of amino, organic, and fatty acid inherited disorders to be profiled using a single blood sample. Further development of allele-specific molecular diagnostics will only cause this issue to continue to evolve. The decision as to which tests to include in mandated newborn screening programs, that is testing performed as a matter of state law on all babies born in that jurisdiction or according to state guidelines in high risk populations, is currently determined by each state, and varies from state to state. This means that no uniformity exists, and the testing done on a newborn in one state, may not match the screening tests performed in a neighboring state. The American Academy of Pediatrics has made recommendations to the federal government to support a national advisory committee to develop uniform, national standards so that each state can properly evaluate which tests should be included in a newborn screening program. The underlying ethical questions only increase when considering the many other issues related to newborn screening.

Incorporation of a test into a screening program traditionally satisfied certain criteria. The test should identify a treatable disorder and any medical intervention should improve the affected individual’s quality of life. That is, the identification of the genetic disorder is of benefit to the child. The disorder should be sufficiently common in a specific or the general population that it constitutes a health risk if left untreated. Other criteria involve the test method’s analytical and diagnostic capabilities. They must be of sufficient quality to provide reliable results in a given population. If the incidence of the disease is low, is it worth screening all newborns or just selected high-risk groups? Should the frequency of positive cases justify inclusion (or exclusion) of a test in the newborn screening program? What of the technological advances allowing for the early identification of untreatable diseases, do these disorders qualify as a public health risk, justifying mandated testing? Should treatment options be a relevant consideration for inclusion in a newborn screening program? Who defines quality of life or benefit? Who decides?

Universal screening (all infants born in that state or territory) exists for PKU and congenital hypothyroidism. Testing for galactosemia is performed in all states except Washington, and 43 states plus the District of Columbia screen all newborns for sickle cell disease. Additionally, six states test for sickle cell disease as part of a selected population or pilot program. Idaho does not offer any screening for sickle cell disease, yet the risk of sickle cell disease is the same as in other states. Case of sickle cell disease testing in Idaho illustrates an interesting scenario easily applied to any inherited disorder. The prevalence of sickle cell disease varies between states due to differences in the ethnic populations located in each geographical area. This fact serves as the rationale for selected states to exclude sickle cell disease, as well as other disorders, in their mandated newborn screening program. In fact, some states have eliminated certain tests because of the infrequency of infants found with the inherited disorder. However, the likelihood of an African-American infant born in Idaho of suffering from sickle cell disease is the same as in other states but an affected infant could suffer grave consequences because of a lack of testing. Inequities and inconsistencies in newborn screening programs between states reflect the decision-making concerns of state policymakers and public health managers. Factors influencing these decisions include the local values of parents, recommendations from professional groups, scientific/medical expertise of lawmakers, and budgetary considerations and restrictions, plus concerns regarding privacy issues. The methods used to make these decisions need to move from the domain of various recommendations and opinions to those based on more empirical data gleaned from analytical studies, clinical trials, research, and collaboration of information between state and state agencies. Some states have responded with pilot programs as a mechanism to collect this data and as a means to explore expanding test menus.

Another basic tenet is that the testing must be affordable and have a sufficiently short turn-around-time so that treatment occurs in a timely fashion before the infant suffers harm. Many state budgetary constraints prevent expansion of newborn screening programs simply because the funding to do so is unavailable. New Jersey recently passed legislation to expand its testing services, but inadequate funding has stalled implementation. If a test is part of a state mandated program, all babies are tested without regard to ability to pay for this service. A newborn screening program consists of five elements: 1) screening; 2) short-term follow-up; 3) diagnosis; 4) treatment and management; and 5) program evaluation and quality assurance. A combination of newborn screening fees, general public health funding, and reliance on third-party payers helps fund the cost of newborn screening programs. Many, but not all (approximately 30) states collect a fee for testing, which include both laboratory and other program services. Of these, 17 states financed more comprehensive services that included follow-up and treatment. Fees may be billed as part of the parents’ medical insurance for allowed newborn care medical coverage, directly to the parents without consideration of healthcare benefits, directly to the primary healthcare professional as part of their fees or services, or as a portion of the delivery charges. Testing fees do not necessarily cover all costs of the newborn screening program. Adequate funding must cover the costs associated with all aspects of the screening program—
FOCUS: NEWBORN SCREENING

pre-analytical, analytical, and post-analytical—from sample collection, transportation, testing, repeat and confirmatory sampling and testing, and parent and healthcare practitioner education to reporting of results. Additionally, the screening program must also address the needs of its population by providing counseling and a system of referral to appropriate medical specialists. Not all children and their parents have health coverage or the means to purchase needed treatment. This is particularly true when costly special formulas, neurodevelopment assessments, therapies, and psycho-social services may not be funded through the patient’s health plan or only for a limited amount of time. In some managed care organizations, the plan requires utilization of in-network professionals without regard to their expertise.

The extent of communication associated with a newborn screening program can be complex. While newborn screen samples may be obtained from a birthing center, mid-wife in the residence, or hospital, most of the testing is performed in private, state, or regional public health laboratories, separate from the birth facility. In the event of repeat sampling and necessary follow-up, the physician ordering the test may not always serve as the infant’s primary healthcare practitioner, particularly in situations where the mother hasn’t identified a pediatrician or a clinic name, or uses a clinic or medical practice where more than one healthcare provider may be in charge of care. The policies surrounding the reporting of results vary widely between states. Inconsistencies exist between states as to the law concerning the reporting authority and/or recipient. In some states, results are provided to the infant’s place of birth, the physician present at birth, and the pediatrician while others only provide reports to the pediatrician, if positive. In these states there’s an assumption of a negative test, when in fact, a baby, for example discharged before 24 hours of age, may have been overlooked. This makes reporting of results and any necessary follow-up extremely difficult. In an attempt to streamline this process and minimize errors, the American Academy of Pediatrics initially suggested that the parent should assign a ‘medical home’ to each child at birth or a short time later. This concept may not only apply to newborn screening but also include immunization records, vision and hearing test results, and other medically important facts. Consensus as to its definition, ramifications such as privacy issues, and responsibilities has yet to be reached by all concerned. The idea of a medical home to provide a systematic reporting and follow-up scheme illustrates the need for a concerted effort between states and a national guideline or system for use in newborn screening programs.

The lack of standardization is not limited to test selection, follow-up, funding, or treatment issues. There exists a great deal of variability in the analytical methods used for screening and confirmatory testing. PKU testing was traditionally screened using a bacterial inhibition assay, known as the Guthrie test, followed by confirmatory testing. While some states still use this method, others screen using automated fluorometric or chemiluminescent assays, while others use tandem mass spectrometry. These differences hold true for other newborn screening tests as well. Each method and testing environment brings with it a lack of uniform cut-off and reference ranges. Testing using similar methods doesn’t mean that the cut-off values for repeat or confirmatory testing are the same between laboratories. As with any laboratory test, the ideal test is one in which a certain level would mean the infant had the congenital defect, while another level would clearly differentiate those who did not. The predictive value of any newborn screening test suffers from the same ambiguity associated with any laboratory test. A repeat test, even when due to a borderline or false positive results, causes a great deal of parental anxiety and requires parental education. An additional dimension in many inherited disorders is that changes in the measured analyte may not be due to a single defect, gene, or even a particular enzyme, further complicating the proper classification of a result. Even when DNA analysis detects a mutation, the scientific information may not prove useful towards treatment or evaluation. Interpretation of results requires specialized expertise, including at least a bachelor’s degree in clinical laboratory science, and a laboratory performing a sufficient quantity of tests. While no universally accepted standard exists, a threshold number of 30,000 samples annually has been suggested. Incorporation of minimum national standards will address the variability currently practiced between newborn screening testing facilities and contribute towards more equitable newborn screening for the more than 400 million babies born annually in the United States and its associated territories.

SAMPLING CONSIDERATIONS

Newborn screening blood samples, taken by heel stick, fill several circles on a filter paper sampling device, forming ‘blood spots’. This device serves several purposes: as the sample requisition and transport device, and as a means of storage for future testing. Blood samples should be collected as late as possible before the infant is discharged, but no later than 72 hours of age. The optimum sampling time in a full-term healthy infant is between 48 and 72 hours of age. Newborn screen samples should always be drawn before a blood transfusion and not before 24 hours of age. Discharging a baby prior to 24 hours of age, necessitates a return trip to the facility to provide a blood sample, which in itself can pose numerous problems for the newborn screening program as well as the parents. In the event the sample is drawn before 24 hours of age, a second sample should be obtained by two weeks of age, but this requirement may vary by state. Infants with low birth weight, born pre-term, treated by antibiotics, or otherwise sick, should be drawn for their newborn screening tests no later than seven days of age.

Since the site of collection and testing most often occurs in different locations, transportation of samples to the testing facility, usually by a courier service or via the postal service, is required. Delivery of samples should occur promptly and samples need to be maintained at an appropriate temperature. The blood spot samples should be kept cool since any heat damage may interfere with the test results.
Once the newborn screening heel stick blood spots are received by the testing laboratory, they are very stable when saved under optimal conditions. Almost all infants screened have residual blood samples, even after repeat testing, and testing laboratories retain these samples. The length of time samples are saved varies between states. For example, ten programs save samples for 21 years or more, six programs for five to seven years, two programs for one to three years, six programs for six to twelve months, and five programs for one to four weeks. Debate as to whether or not these residual samples should be used for later biochemical, forensic, or genetic analyses exists, but these samples remain a rich resource for future analytical, epidemiological, environmental, and clinical research. Residual samples have already been used in numerous validation studies. Another use may be in forensic testing, since the heel stick blood sample may be the only verifiable biological sample for some children. Testing may be essential in postmortem identification of a genetic condition associated with the child’s death. One state has decided to store newborn blood spots indefinitely to permit identification of children who are kidnap victims. These ideas bring up a host of separate ethical considerations. Residual samples fall into two major classifications: identifiable (can be linked to personal information) or unlinked (anonymous). Some topics for debate include consent for research or for purposes other than newborn screening, issues of privacy and confidentiality, or even whether human biological samples should be used for research purposes at all. Another persistent ethical issue in newborn screening is whether screening is voluntary or mandatory. A voluntary approach requires informed consent for testing. The potential advantage includes more prompt and efficient communication in cases requiring follow-up or for possible incorporation in experimental or pilot programs. A mandatory approach requires parents to object to and often refuse testing, without fully understanding the implications of that refusal. Only 13 states require that parents be informed before testing takes place. Strategies, guidelines, and legislation governing these issues will need to be developed in the near future.

ADVANCES IN SCREENING METHODS
Over the past 40 years, technology has allowed the medical community to recognize hundreds of disorders linked to inherited metabolic defects, even in asymptomatic individuals. The expansion of molecular testing and DNA screening, along with the completion of the Human Genome project, will further provide laboratorians with the tools necessary for specific identification of existing and newly identified genetic disorders. More recently, the use of non-isotopic immunoassays and tandem mass spectrometry (MS/MS) allows fast detection of an increasing number of tests performed on a single sample. In several states, the availability of MS/MS allows more than 30 different enzyme deficiencies to be included as a part of the universal newborn screen. The number continues to grow.

Mass spectrometry, paired with gas or liquid chromatography, has been a valuable analytical tool since the mid-1960s. Early on, the expense and required maintenance limited its utility in routine clinical settings. Advances in technology and streamlined models currently make this technology feasible for more facilities and further expansion of testing. The basic principles remain the same, in that a mass spectrometer measures the mass-to-charge (m/z) ratio of ionized molecules. Inherited metabolic diseases involve some abnormality of an amino acid, organic acid, fatty acid, or entire protein. These molecules are easily charged or ionized (z) either directly or through suitable derivatization. In traditional mass spectrometry, the initial step in the process requires isolation of

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**Figure 1. Tandem mass spectrometry (MS/MS)**

1. **Complex molecule**
2. **Mass Analyzer 1**
3. **Fragmentation & Ionization**
4. **Database Search & Identification**
5. **Mass Analyzer 2**
6. **Mass charge (m/z)**
7. **Mass/charge (m/z)**

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the component of interest using time-consuming liquid or gas chromatography. The stream then flows through a mass analyzer and identification is made based on the fragmentation pattern. While chromatographic processes may still be coupled to MS/MS, they limit the number of compounds that can simultaneously be analyzed with a single mass analyzer and only compounds that are or can be made volatile are feasible.

MS/MS incorporates two or more mass spectrometers (the original idea behind the term ‘tandem’—whether in space or time) separated by a collision chamber. Compounds are isolated using one mass analyzer according to their m/z ratio. Further fragmentation of the sample in a collision chamber (daughter ions) followed by subsequent mass analysis provides the laboratorian with an ion spectrum of specific structural information for that generation of fragment ions in a matter of minutes (Figure 1). Sequential searching of the daughter ions enables analyses of many structurally related substances from a given blood spot sample, making it an ideal newborn screening tool. Compounds in fractions of nanomoles can be separated and identified from one sample and the process, from start to finish, takes about three hours. To ease identification, samples may be derivatized, such as the interaction of blood organic acids with butanolic HCl (butylation) prior to analysis resulting in readily identifiable daughter ions. The entire process is automated. The ionization process has undergone significant modifications, so that ‘softer’ methods using lower energies cause less fragmentation of the target molecules, making it ideal for use with amino acids, peptides, and organic acids. Even when using this method, confirmation of a positive or inconclusive result remains necessary, often using DNA testing.

INHERITED METABOLIC DISORDERS
Inherited metabolic disorders, also known as in-born errors of metabolism, interrupt some aspect of a metabolic pathway changing the concentration of precursors or altering the function of physiologically important compounds, such as transport or carrier proteins. This may be due to a pathway enzyme that is either absent or one in which there is altered activity resulting in a buildup of some intermediate metabolite above the expected range and may serve as the basis for many newborn screening tests. It may be the absence of an appropriate product or insufficient substrate that is the underlying etiology of the disorder, not the abnormally high level of metabolite that is measured as a biochemical marker of the disease.

An early difficulty in diagnosing inherited metabolic disorders was associated with the fact that clinical manifestations are not always readily apparent. The most severely affected newborns may present with clinical symptoms, including unexplained failure to thrive, coma, or even death, within one week of life. However, some clinical symptoms delay until the infant’s system is stressed, as with a first earache, fasting, or a change in diet, puberty, or adulthood. It should be noted that, at the time, there was little understanding of inherited diseases and the incidence of those that were recognized was considered quite rare.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotinidase deficiency</td>
<td>1:60,000-125,000</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td>1:12,000-15,000</td>
</tr>
<tr>
<td></td>
<td>1:300 Yupik Eskimos</td>
</tr>
<tr>
<td>Congenital hypothyroidism</td>
<td>1:4,000</td>
</tr>
<tr>
<td></td>
<td>1:2,700 Hispanic</td>
</tr>
<tr>
<td></td>
<td>1:700 Native Americans</td>
</tr>
<tr>
<td>Congenital toxoplasmosis</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>1:2,500</td>
</tr>
<tr>
<td>Fatty acid oxidation disorders</td>
<td></td>
</tr>
<tr>
<td>Medium chain</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Long chain</td>
<td>1:50,000</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>1:1,600,000-80,000</td>
</tr>
<tr>
<td>Hemoglobinopathies*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:58,000</td>
</tr>
<tr>
<td></td>
<td>1:400 African Americans</td>
</tr>
<tr>
<td></td>
<td>1:1,100 Hispanics (Eastern States)</td>
</tr>
<tr>
<td></td>
<td>1:2,700 Native Americans</td>
</tr>
<tr>
<td></td>
<td>1:11,500 Asian Americans</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>1:80,000-100,000</td>
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<tr>
<td></td>
<td>1:760 (Mennonite population)</td>
</tr>
<tr>
<td>Hyperphenylalanemias (including PKU)</td>
<td>1:10,000-15,000</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>1:200,000</td>
</tr>
<tr>
<td>Organic acidemias†</td>
<td>1:30,000-50,000</td>
</tr>
</tbody>
</table>

* Sickle cell disease
† Incidence data varies
Table 1 provides estimated incidence of selected disorders in the United States. Disorders involved in infant mortality went unrecognized as genetic defects for many years, particularly when the presenting signs and symptoms lacked a link to any known metabolic pathway deficiencies, or went unsuspected because the parents and any siblings were healthy. In addition, the signs and symptoms in metabolic diseases may appear non-specific, vary in their expression, or be attributed to more common disorders. Clinical symptoms include metabolic acidosis; hypoglycemia; cardiac, hepatic, and renal failure; cataracts; mental retardation; seizures; skin rash; Fanconi’s syndrome; poor feeding; irritability; constipation and vomiting in infants; adrenal crisis (salt wasting); shock; ambiguous genitalia; developmental delays; and sudden infant death. This array of findings may not be present in all disorders.

Newborn screening testing is routinely performed in specialized private or public health laboratories, and for many scientists, the screening tests and the associated disorders often remain an academic discussion. Most curricula include discussions of phenylketonuria (PKU), galactosemia, cystic fibrosis, maple syrup urine disease, congenital hypothyroidism, and the hemoglobinopathies. More recently, additional screening for metabolic disorders include congenital adrenal hyperplasia, biotinidase deficiency, homocystinuria, fatty acid oxidation defects, organic acidemias, and a variety of amino acidemias. Universal screening (all 50 states plus the District of Columbia) exists for classical PKU and congenital hyperthyroidism. MS/MS is used in 15 states in selected populations or as part of pilot programs. North Carolina possesses the most comprehensive mandated panel to date. Table 2 provides a listing of the specific tests performed in each state as of May 2002, with an overall summary in Figure 2. The best resource for the most up-to-date offerings is the respective state’s Department of Health.

SELECTED DISORDERS

Phenylalanemia
PKU, an autosomal recessive disorder, is caused by either a gene mutation causing a deficiency or absence in the activity of the enzyme phenylalanine hydroxylase, an enzyme responsible for the breakdown of phenylalanine to tyrosine. This is known as classical PKU. This results in an elevation of phenylalanine but a decrease in blood tyrosine levels, both of which can be used for PKU screening. Tyrosine and its associated metabolic enzymes synthesize the important neurotransmitters, serotonin and dopamine, so a decrease has many adverse effects. Co-factor variants that also result in hyperphenylalaninemia, may cause progressive neurologic deficits, even early death. Elevated phenylalanine impairs the early development of the central nervous system, and in untreated individuals, causes mental retardation. Convulsions, eczema, autistic-like behavior, and hyperactivity are associated findings. Since phenylalanine is an essential amino acid (obtained from the diet), treatment for classical PKU involves dietary restriction of this amino acid before the age of four weeks for the most effective treatment. The co-factor variation requires a special modified diet, low in phenylalanine but supplemented with the identified missing co-factors. Maternal PKU is problematic since high blood levels of phenylalanine are teratogenic to the fetus.

Congenital hypothyroidism
Congenital hypothyroidism results from an inadequate production of thyroid hormone due to a number of different causes. In addition to the metabolic symptoms associated with hypothyroidism, patients who go undiagnosed suffer from mental retardation, neonatal jaundice, and variable degrees of growth failure, deafness, and neurologic problems. The clinical signs may not develop until the infant is several months of age or older and the most effective treatment, oral levothyroxine, should begin within the first few weeks of life. Treatment of congenital hypothyroidism has proven highly successful.

Galactosemia
Galactosemia results in elevated galactose, a monosaccharide component of the carbohydrate lactose found in cow’s milk. Many commercial baby formulas contain lactose, while some of the soy-based formulas do not. The elevation of galactose is attributed to defect in the conversion of galactose to usable glucose-1-phosphate. This process occurs by several metabolic steps, and an enzyme defect in any of them can cause an elevation of galactose. Galactosemia can lead to hypoglycemia, failure to thrive, vomiting, liver disease, cataracts, and mental retardation. Symptoms can occur before the result of the newborn screen is received, but only after ingestion of galactose. At very high risk are bottle-fed babies using lactose based formulas. It is usually fatal, often associated with complications from liver failure, sepsis (E. coli), or bleeding, if left untreated. In treated survivors, complications include lowered IQ, which seems dependent on the time of treatment—the earlier, the better—and learning disabilities. Treated females often experience ovarian failure, or secondary amenorrhea. Complete dietary exclusion of galactose, including but not limited to lactose containing products, must be maintained for life.

Hemoglobinopathies
Hemoglobinopathies represent a group of inherited disorders due to abnormalities in hemoglobin. In newborn screening programs, states may only test for sickle cell disease, which for homozygotes, causes sickle cell anemia. In this disorder, valine is incorrectly substituted for glutamine in the sixth position of the beta chain of the hemoglobin molecule causing an increase in the red cell’s fragility, particularly in low oxygen environments, resulting in hemolysis and vascular occlusions. The identification of additional hemoglobinopathies and/or thalassemias, as part of a newborn screen, varies between states.

Congenital adrenal hyperplasia
Congenital adrenal hyperplasia (CAH) consists of a family of disorders arising from defects in the enzymes needed for the synthe-
FOCUS: NEWBORN SCREENING

Table 2. U.S. newborn screening status

<table>
<thead>
<tr>
<th>State</th>
<th>CAH</th>
<th>SCD</th>
<th>MSUD</th>
<th>BIO</th>
<th>HCY</th>
<th>FOD</th>
<th>OAD</th>
<th>OAA</th>
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<td>California</td>
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<td>V(^{10})</td>
<td>V(^8)</td>
<td>V(^5)</td>
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Note: All locations listed provide universal testing for PKU and congenital hypothyroidism; all except Washington perform galactosemia. These tests have not been included in this table.

\(^1\) Based on composite of U.S. National Screening Status Report (INNSGRIC) and Title V Block Grant FY 2000 Annual Report and FY 2002 Application, Health and Human Services Administration, Maternal and Child Health Bureau. Precise status for each area should be confirmed by contacting the local Department of Public Health.

\(^2\) Mandated by law but not yet funded.

\(^3\) State law requires that testing be offered, but since it is not mandated, considered here as voluntary.

\(^4\) Supplemental testing offered; BIO = biotinidase deficiency; CAH = congenital adrenal hyperplasia; CF = cystic fibrosis; FOD = fatty acid oxidation disorders; G-6-PD = glucose-phosphate dehydrogenase deficiency; HCY = homocystinuria; MSUD = maple syrup urine disease; OAD = other amino acidemias (ASA = arginosuccinate lyase deficiency; CIT = citrullinemia); OAA = organic acidemias; P = universal pilot program; R = mandated for all newborns; SCD = sickle cell disease; TOX = congenital toxoplasmosis; TYR = tyrosinemia; V = selected populations, pilot study, or as requested.

1, 2, 3. Disorders listed in these are tested using MS/MS and the superscript in these categories indicate the number of disorders included.

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sis of adrenal corticosteroid hormones. The biosynthesis of adrenal corticosteroids begins with adrenal corticotrophic hormone (ACTH) that acts on cholesterol, giving rise to pregnenolone. Pregnenolone, through the action of different enzymatic pathways, produces the mineralocorticoids (aldosterone), glucocorticoids (cortisol), androgens (testosterone), and the estrogens. The clinical consequences of the deficiency arise from the: 1) overproduction and accumulation of precursors prior to the specific blocked enzymatic step, and 2) magnitude of the hormone deficiency. Almost 90% of all cases involve a defect in 21-hydroxylase (21-OH), an enzyme needed for proper production of the mineralocorticoids (aldosterone) and glucocorticoids (cortisol). Other enzymes commonly linked to this disorder include 17-alpha hydroxylase, required for cortisol and androgen production, and 11-beta-hydroxylase needed for proper cortisol production. Because of a 21-OH defect, progesterone precursors accumulate, with some diverted to the androgen pathway. In addition, the negative feedback cortisol normally exerts on ACTH via the pituitary is impaired, causing increased ACTH production.

In utero, female fetuses exposed to excess androgens show masculinization and ambiguous genitalia at birth while male fetuses appear normal. Lack of aldosterone causes loss of sodium (hyponatremia), accumulation of potassium (hyperkalemia) which may result in a life-threatening salt-wasting syndrome, increased renin levels associated with hypovolemic shock, and decreased cortisol producing hypoglycemia. Early treatment of infants to correct the electrolyte and glucose levels can ameliorate these life-threatening symptoms and prevent incorrect sex assignment. In the absence of salt-losing phenomena, CAH may not be diagnosed until three-to seven-years of age, particularly in boys, when accelerated bone maturation that ultimately leads to short stature is detected. In milder forms, this condition can cause hirsutism and menstrual irregularities in women. Administration of glucocorticoids inhib-
its excessive production of androgens, prevents progressive virilization, and stabilizes renin levels. Adjusting the dose during times of stress, such as surgery or infection, may be necessary. The incidence in the general population of 21-OH deficiency is found to be about 1:14,000 births but with a much greater incidence in Yupik Eskimo populations where it increases to 1:680 births.

**Maple syrup urine disease**

Maple syrup urine disease (MSUD) is a rare disorder (1:200,000 births) due to a defect in the mitochondrial enzyme branched-chain amino acid dehydrogenase complex. Leucine, isoleucine, and valine are branched essential amino acids that are catabolized to corresponding keto acids through the process of transamination. A branched-chain keto acid dehydrogenase enzyme complex consisting of four proteins catalyzes the oxidative decarboxylation (removal of CO₂) of the resulting keto acids. Cofactors required by this complex include Mg²⁺, thiamine pyrophosphate, Co-enzyme A, FAD, and NAD⁺. The catabolism of leucine, isoleucine, and valine after the branched chain amino acid carboxylase reaction yields acyl-CoA thiosteres (acetoyacetate and acetyl-CoA; propionyl-CoA; acetyl-CoA, and succinyl-CoA-respectively) that serve as important lipid and carbohydrate metabolic pathway substrates. MSUD derives its name from the characteristic odor, reminiscent of maple syrup, of the urine from these patients. There are five different variants of this disease presenting an array of clinical symptoms. The most severe form, that of classic MSUD associated with the absence of branched-chain alpha-keto acid decarboxylase, results in accumulation of keto acids and their associated amino acids causing severe acidosis in the first ten days of life which, if undetected and untreated, is usually lethal in the first month. Initial symptoms may be poor eating, lethargy, and coma. Variant forms of MSUD differ in clinical presentation, depending on the severity and cause of the enzyme deficiency, and the age at diagnosis. Treatment includes lifetime dietary restriction of branched-chain amino acids and in the thiamine deficient forms, treatment with thiamine, but neurologic symptoms, including mental retardation, may persist even after treatment.

**Homocystinuria**

Homocystinuria represents a group of disorders related to an enzyme defect in some aspect of the interrelated catabolism of the sulfur containing amino acids (methionine, cysteine, and homocysteine). Like other inherited disorders, the severity depends on where and to what degree in the pathway the defect occurs. Methionine, linked to intermediates derived from the breakdown of folate with vitamin B₉ and B₁₂ as cofactors, forms homocysteine which is further metabolized to cysteine and α-ketobutyrate. The pathway from methionine to homocysteine is reversible and low levels of homocysteine are normally reconverted to methionine as a mechanism to conserve sulfur. Problems may arise with the enzyme responsible for catalyzing the reaction of homocysteine to cysteine or with the ability to properly utilize folate and the B vitamins.

The first causes elevations of both methionine and homocysteine, while the latter only homocysteine, which is diagnostic, if the screening program evaluates both substances. In either case, elevated homocysteine levels lead to homocystinuria plus serious complications that develop with age. Side effects include ocular lens problems, skeletal abnormalities, and the more serious complications attributed to arterial or venous thrombosis that may lead to an early death. Some individuals die of thrombolytic complications within the first year of life but approximately 50% of untreated individuals die by 25 years of age. Treatment depends on the source of the defect, but some individuals respond to vitamin treatment, and those non-responsive to this treatment, require specialized diets restricting methionine but supplemented with cysteine.

**Biotinidase deficiency**

Biotinidase deficiency testing occurs in more than twenty U.S. territories. This enzyme liberates the vitamin, biotin, to a form usable in the many dependent carboxylase (CO₂ fixing) reactions. These include enzymes involved in fatty acid synthesis, gluconeogenesis, and leucine metabolism. As part of a multi-step process, biotin becomes linked to the active site in the carboxylase enzyme and this complex is called biocytin. Biotinidase is an enzyme that cleaves the link between the enzyme and biotin. During normal catabolism, protease enzymes break down carboxylase enzymes and reclaim the associated amino acids and biocytin for recycling. Individuals with biotinidase deficiency can't regenerate biotin from biocytin leading to multiple carboxylase deficiency. Affected individuals initially show combinations of neurologic and cutaneous findings, including seizures, dermatitis, alopecia, conjunctivitis, hearing loss, developmental delay, and in some instances, acute metabolic decompensation resulting in coma. Death may occur. Early treatment with biotin can prevent clinical consequences but delayed treatment may not prevent some of the less severe consequences.

**Fatty acid oxidation disorders**

One of the latest groups of disorders added to newborn screen menus, in a large part due to MS/MS capability, is that related to enzyme deficiencies of fatty acid oxidation. Fatty acid oxidation occurs in the mitochondria, after the fatty acid is transported from the cell cytoplasm to the mitochondria via carnitine. It continues as a series of controlled processes resulting in the breakdown of fatty acids for energy, particularly during intense exercise and the fasting state, by the liver, heart, and skeletal muscles. β-oxidation occurs as an acyl-CoA (fatty acid) dehydrogenase enzyme complex and initially removes two carbon units from an activated (CoA attached) fatty acid decreasing the original fatty acid by two carbons. This shortened fatty acid subsequently goes through repeated ‘cycles’ until the final production of two molecules of acetyl CoA (even numbered fatty acids). Structurally, fatty acids, derived from the diet or stored triglycerides in adipose tissue, contain a polar ‘head’ (–COOH) attached to repeating carbon units (CH) that make up the fatty acid ‘tail’ of varying length. There are different acyl-CoA dehydrogenases em-

**FOCUS: NEWBORN SCREENING**

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- **There are different acyl-CoA dehydrogenases em-**
FOCUS: NEWBORN SCREENING

employed in fatty acid oxidation based on the length of the fatty acid involved. They are short-chain (SCAD), medium-chain (MCAD), long-chain acyl-CoA (LCAD), long-chain-hydroxy- (LCHAD), and very-long-chain (VLCAD) acyl-CoA dehydrogenase. They all operate in the same general manner.

In addition to the acetyl-CoA produced, each cycle of β-oxidation, via FAD, and NAD⁺, shuttles electrons to the electron transport system (ETS/respiratory chain) as a mechanism for more direct ATP production. A defect in one of these enzymes, from activation through the complete process of fatty acid catabolism, can cause a fatty acid oxidation disorder. Other inherited enzyme defects in the transfer of electrons to the ETS can cause increased levels of organic acids, and while related to proper fatty acid catabolism, are classified as organic acidurias. Clinical symptoms of fatty acid oxidation defects reflect the enzyme deficiency involved, but may be induced by fasting. Symptoms include lethargy, hypoglycemia, metabolic acidosis, failure to thrive, coma, and even death. MCAD, with the highest incidence found in the Caucasian population of northern European descent, may lead to death in 20% to 25% of those affected. It is estimated that 1 in 100 sudden infant death syndrome deaths is probably the result of MCAD.²¹ Here again, data gleaned from newly instituted screening, whether mandated or as part of a pilot program, are sorely needed and beg the ethical issues raised earlier. Treatment includes avoidance of fasting and medium-chain fatty acids. Supplemental carnitine may also prove useful.

CONCLUSION

The selected issues and disorders discussed as part of this review serve only to highlight some of the many facets of newborn screening. Some states already include tests for defects such as cystic fibrosis, tyrosinemia, and glucose-6-phosphate dehydrogenase deficiency, plus many of the amino acid and organic acidurias not specifically addressed here. Advances in newborn screening, including identification of the exact genetic mutation, make the possibility of including the biochemical markers for many additional inherited defects very feasible. The ethical issues involved and the lack of nationally standardized models for many aspects of this field prove challenging. These questions will only become more complex as testing methods continue to improve.

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Newborn Screening: 
New Developments in a Proven Field

ROGER B EATON, ANNE MARIE COMEAU, THOMAS H ZYTKOVICZ, CECILIA LARSON

ABBREVIATIONS: CF = cystic fibrosis; IRT = immunoreactive trypsinogen; MCAD = medium chain acyl-CoA dehydrogenase deficiency; MS/MS = tandem mass spectrometry; MSUD = maple syrup urine disease; NENSP = New England Newborn Screening Program; PKU = phenylalaninemia.

INDEX TERMS: newborn diseases; newborn testing; testing.


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FOCUS: NEWBORN SCREENING

The field of newborn screening is dynamic, incorporating new technical developments that can improve the quality of life for more babies than ever before. Although 'newborn screening' includes screening for hearing and other disorders, in this article newborn screening will refer to public health-regulated testing of blood samples from newborns. This article on newborn screening provides opportunities to: 1) consider how the purpose of a medical test, e.g., diagnostic vs. screening, relates to assay performance measures; 2) consider newborn screening as a model for the application of laboratory science to public health; and 3) review some recent technical developments in the field.

'SCREENING’ – RELEVANCE TO THE LABORATORIAN

In designing testing algorithms and laboratory cut-offs, it is important to consider the purpose of the testing and the likely population from whom samples will be received. The primary purpose of a newborn screen test is to select from the general population of newborns (where the probability of the disorder is very low) a manageable number of babies with significantly higher risk for the targeted disorder. The purpose of a test applied to clinically presenting patients (where the prior probability for the disorder is already much greater than for the general population) is to further refine the risk for the disorder, or to establish the diagnosis.

Let us consider the application of a test with a 90% sensitivity and a 99% specificity to each of the above two situations (Table 1). In the clinical diagnostic situation, the purpose of the test is to aid the clinician in defining a diagnosis. The clinician utilizes the expertise of the laboratory to add one more piece of information to the signs, symptoms, and other laboratory results that comprise the complete clinical picture. The clinical signs and symptoms themselves are part of a selection procedure, so that the patient on whom an appropriate diagnostic test is ordered typically has a much...
FOCUS: NEWBORN SCREENING

higher chance of having the disorder than a healthy member of the general population. While the prevalence of the disorder under consideration in the general population may be 1:12,000, the clinical presentation alone may bring the risk for the disorder in the range of one in ten. If the physician is confronted with 100 clinically presenting patients, then one would expect that ten will actually have the disorder, that nine out of these ten patients with the disorder will be correctly identified by the laboratory with a positive test, and only one of the 90 unaffected individuals will yield a (false) positive test. Though the test did not perform perfectly, the patient is already in the hands of a health professional, who will take into account all components of the clinical picture when forming the diagnosis. Conflicting information may even prompt a re-test.

By contrast, consider the use of a test, with similar performance characteristics, for the purpose of newborn population screening. The test is applied to an unselected population with a prior risk of 1:12,000. The laboratory of the New England Newborn Screening Program (NENSP) receives specimens from 140,000 newborns each year for phenylalanine testing, designed to screen for PKU with a prevalence of 1:12,000. It can be readily calculated that on average, about 12 babies a year will have PKU. A test with 90% sensitivity will detect 11 of those babies (potentially leading to a false sense of security for the clinician or family). Careful consideration needs to be given to which disorders are included on the newborn screening panel, and so each state has developed its own program.1 Typically these criteria include the availability of a low cost test with adequate sensitivity, specificity, and throughput. In addition, for a disorder to be included as part of a public-health regulated population-based newborn screening initiative, the infant being screened should benefit from the screen, the disorder should be significant enough in severity and frequent enough in the population to warrant all individuals being tested, and an effective treatment should be available and accessible to the general population. In practice, many disorders meet some but not all criteria, and so each state has developed its own process to determine which disorders should be added to a newborn screening panel.

Meanwhile, what is the impact of the seemingly strong 99% specificity? In our screened population, 1,400 babies without PKU will yield a positive screen. The positive screen will require at least some follow-up testing, most likely accompanied by at least some (and perhaps significant) levels of anxiety on the part of the family. Also, particularly when one realizes that the NENSP now offers screening for 30 disorders, the medical community is burdened by follow-up testing that, if not kept to a minimum, may lead to a “cry wolf” scenario, diluting the impact of the truly important notifications of out-of-range tests in newborns with critical disease. Raising the sensitivity of the assay from 90% to 99% by lowering the cutoff (so as not to miss affected babies) will be associated with an even worse specificity. Clearly the performance demands on tests used for population-based screening must be high.

The analyst in the newborn screening laboratory has the challenge to choose or develop a blood test that: 1) will detect the targeted disorder in blood samples typically taken between one and four days of age; 2) has high sensitivity; 3) has extraordinarily high specificity; 4) can be applied to hundreds of samples every day; and 5) can do all of this at reasonable cost. Finally, for newborn screening to be effective, the laboratorian also needs to understand the role of the laboratory within the context of the public health goals.

NEWBORN SCREENING AS PUBLIC HEALTH

The goal of newborn screening is to detect an unsuspected disorder before significant and irreparable harm comes to the baby, and to initiate intervention that will prevent the development of that harm. When a laboratory test for such a disorder becomes available, the disorder becomes a candidate to be added to a newborn screening panel.

The decision to test all babies born in a state for a panel of medical tests is a significant one. Screening requires resources, both for the initial testing and for follow-up diagnostic testing and evaluations; screening may produce positive results in normal babies (resulting in anxiety and perhaps additional testing and evaluation due only to the screen), and screening may yield negative results in some affected babies (potentially leading to a false sense of security for the clinician or family). Careful consideration needs to be given to which disorders are included on the newborn screening panel, and so criteria have been established for evaluating which disorders warrant screening. Typically these criteria include the availability of a low cost test with adequate sensitivity, specificity, and throughput. In addition, for a disorder to be included as part of a public-health regulated population-based newborn screening initiative, the infant being screened should benefit from the screen, the disorder should be significant enough in severity and frequent enough in the population to warrant all individuals being tested, and an effective treatment should be available and accessible to the general population. In practice, many disorders meet some but not all criteria, and so each state has developed its own process to determine which disorders should

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<tr>
<td>Number without disorder with positive test</td>
</tr>
<tr>
<td>Performance characteristics of test: sensitivity = 90%; specificity = 99%</td>
</tr>
</tbody>
</table>

240 VOL 15, NO 4 FALL 2002 CLINICAL LABORATORY SCIENCE
be included as part of its population-based screening. Since the number of considerations is immense, this public health policy decision is a rich example of how policy emerges from the interface of laboratory science, ethics, economics, and politics.2

Another important aspect of the public health nature of newborn screening is that the laboratory analysis is only one link in the chain of responsibilities that must function effectively for the goals of newborn screening to be realized. A recent overview of the various activities that need to be integrated into comprehensive newborn screening was presented in a special issue of Pediatrics (2000).1 The newborn screening laboratory shares with the diagnostic laboratory the responsibility to accurately test samples, and report results with appropriate interpretations. However, even the most accurate laboratory fails to meet the goals of newborn screening if a sample is never taken from the baby; if the sample is not transported to the laboratory in a timely manner; if the result is only reported to the hospital that sent the specimen and the current healthcare provider is unaware of the result; and if critical reports are not communicated along with enough supporting communication to assure timely engagement of the baby with appropriate specialists. Although these connections can be performed under one of several models, many would agree that orchestration of the multiple responsibilities is best performed by integrating the various responsibilities under only one or two administrative bodies.

The model favored by Massachusetts was to bring all operative responsibilities under one administrative body, which is the New England Newborn Screening Program (NENSP), a program of the University of Massachusetts Medical School. The newborn screening program itself forms the glue that integrates the many links.

Table 2. Evolution of newborn screening prior to recent expansion

<table>
<thead>
<tr>
<th>Disorder targeted by newborn screening</th>
<th>Year implemented in Massachusetts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>1962</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>1963</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>1964</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>1968</td>
</tr>
<tr>
<td>Congenital hypothyroidism</td>
<td>1976</td>
</tr>
<tr>
<td>Congenital toxoplasmosis</td>
<td>1986</td>
</tr>
<tr>
<td>Sickle cell</td>
<td>1990</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td>1990</td>
</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>1992</td>
</tr>
</tbody>
</table>

Recent expansion of the newborn screening panel

In February of 1999, the number of disorders offered for screening in Massachusetts expanded from nine to 30 (Table 3). The process of the policy development has been detailed elsewhere.2 The expansion incorporated the application of two very separate sets of technologies, which will now be reviewed separately.

Newborn screening for cystic fibrosis

Newborn screening for cystic fibrosis (CF) became a possibility with the recognition in 1979 that most babies with CF have elevated blood levels of immunoreactive trypsinogen (IRT).4 The specificity for CF of performing an IRT assay on a single newborn sample is poor. However, the identification of the gene (CFTR) responsible for CF in 1989 paved the way for a two-tier approach to CF screening, with improved specificity and shorter turn-around time to diagnosis.5 The assay for elevated IRT (first tier) followed by a test for the common mutation (D508) of the CFTR gene (second tier) proved to be appropriate. This approach was implemented in the US by 1991.6

By 1998 six states (CO, CT, MT, PA, WI, and WY) screened some or all babies for CF. However, definitive documentation of benefit of newborn screening in the long term pulmonary outcome remains elusive. For this reason, CF screening was implemented in Massachusetts as a pilot study. Details of the implications of implementing screening for a disorder as a pilot, rather than as a mandate, are beyond the scope of this overview. It is relevant to note that pilot testing required that parents be given the option to have their baby screened or not screened. This required educational materials to be developed and distributed to each parent. It is notable that over 98% of all babies born in Massachusetts since the pilot testing began have been tested for all pilot tests.

Massachusetts adopted the two-tier screening approach, with significant enhancements to the second tier relative to previous population-based screening approaches.
FOCUS: NEWBORN SCREENING

The first tier is a test of the newborn blood spot for IRT. A defect in the CFTR gene interferes with normal transport of ions across cell membranes. One result of this is the well-known thickening of pulmonary secretions, generally developing later in life. It is believed that a similar thickening of secretions in, and blockage of, the pancreatic ducts causes the leakage of trypsin precursors into the blood stream, and it is these molecules that are detected by IRT assays. The NENSP utilizes a time-resolved fluoroimmunoassay kit (DELFIA Neonatal IRT, Perkin Elmer Life Sciences) for measuring IRT. To microtiter wells containing solid-phase bound monoclonal anti-IRT antibodies are added discs punched out of the newborn dried blood sample (source of IRT), and an elution buffer containing europium-labeled monoclonal antibodies directed to a second antigenic determinant on IRT. After suitable incubation, the sandwich is washed and enhancement solution is added. The enhancement solution dissociates the europium ions from the labeled antibody, and these ions form a highly fluorescent chelate with components of the enhancement solution. The degree of fluorescence corresponds to the amount of IRT in the specimen. Kit standards provide five levels of IRT as calibrators, plus a negative. The kit contains two levels of controls on dried filter papers. The testing laboratory also prepares two levels of in-house controls, using sera from selected individuals with high amylase levels pre-screened for high IRT levels, diluted appropriately in normal human serum, mixed with washed human RBC, and applied to filter paper to form dried blood spots that mimic those received from newborns. Those samples from a run (minimum run size = 300 samples) that yield the highest 5% of IRT results are selected and to these the second tier test is applied.

The 5% of specimens yielding the highest IRT results prompt DNA testing (on the same specimen) for mutations of the CFTR gene by PCR based amplification followed by linear array detection. Rather than testing only for the common D508 gene, the NENSP tests for 27 prevalent mutations of the CFTR gene.

Samples that were selected for the second tier (prompted by IRT) and are shown to have one (category ‘B’) or two mutations (category ‘C’) are considered to have a positive CF screen. In addition, babies whose first tier yielded an IRT test in the upper 0.2% (even without any of the 27 mutations tested) are considered to have a positive CF screen (category ‘A’). Physicians of these babies are notified in the context of a consultation listing the likelihood of the baby to have CF (according to experience-based statistical associations of the various categories with final diagnosis), and the babies are referred to a CF center for measurements of electrolytes in the baby’s sweat (sweat test). The sweat test is the gold standard diagnostic test, and will clarify for the family whether or not the baby has CF. Genetic counseling is provided by the CF center or its associates.

CF screening began in Massachusetts in February of 1999. During the first three years of testing, 236,988 babies were screened for CF, and 99.5% of babies yielded a negative screen. Among the remaining 1,153 babies who were referred to CF Centers for sweat testing, 86 were found to have cystic fibrosis. These babies have had the opportunity for enzyme treatments to help overcome the nutritional deficits known to occur in untreated babies with CF, and to be monitored for early signs of pulmonary infection. It is hoped that this care will be shown to improve the long term pulmonary outcome for these babies as they grow into adulthood.

Newborn screening by tandem mass spectrometry

The remaining 20 additional disorders implemented in February of 1999 as part of the expanded screening are all detected

Table 3. Expanded newborn screening: new disorders offered after February 1, 1999 to all babies born in Massachusetts

<table>
<thead>
<tr>
<th>Group</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandated</td>
<td>MCAD [Medium-chain acyl-CoA dehydrogenase deficiency]</td>
</tr>
<tr>
<td>Pilot 1</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>Pilot 2</td>
<td>ARG [Argininemia]</td>
</tr>
<tr>
<td></td>
<td>ASL [Argininosuccinic aciduria]</td>
</tr>
<tr>
<td></td>
<td>ASS [Citrulinemia]</td>
</tr>
<tr>
<td></td>
<td>CPT II [CPT II deficiency]</td>
</tr>
<tr>
<td></td>
<td>GA I [Glutaric acidemia I]</td>
</tr>
<tr>
<td></td>
<td>GA II [Glutaric acidemia II]</td>
</tr>
<tr>
<td></td>
<td>HHH [HHH syndrome]</td>
</tr>
<tr>
<td></td>
<td>HMG [HMG-CoA Lyase deficiency]</td>
</tr>
<tr>
<td></td>
<td>IVA [Isoleucineria]</td>
</tr>
<tr>
<td></td>
<td>LCAD [long-chain acyl-CoA dehydrogenase deficiency]</td>
</tr>
<tr>
<td></td>
<td>LCHAD [long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency]</td>
</tr>
<tr>
<td></td>
<td>MCC [ß-Methylcrotonyl carboxylase deficiency]</td>
</tr>
<tr>
<td></td>
<td>MMA [Methylmalonic aciduria]</td>
</tr>
<tr>
<td></td>
<td>PA [Propionic acidemia]</td>
</tr>
<tr>
<td></td>
<td>SCAD [short-chain acyl-CoA dehydrogenase deficiency]</td>
</tr>
<tr>
<td></td>
<td>ß-KT [ß-Ketothiolase deficiency]</td>
</tr>
<tr>
<td></td>
<td>Tyr I [Tyrosinemia I]</td>
</tr>
<tr>
<td></td>
<td>Tyr II [Tyrosinemia II]</td>
</tr>
<tr>
<td></td>
<td>VLCAD [very-long-chain acyl-CoA dehydrogenase deficiency]</td>
</tr>
</tbody>
</table>

The remaining 20 additional disorders implemented in February of 1999 as part of the expanded screening are all detected...
by tandem mass spectrometry (MS/MS). One of these disorders, medium chain acyl-CoA dehydrogenase deficiency (MCAD), was added as a mandate, joining the nine mandated disorders already on the Massachusetts panel. The other 19 disorders were added as a second pilot study, and so parents are given the same opportunity to choose whether they want their babies screened for these pilot disorders. Again, over 98% of all babies born since February of 1999 have been tested for all pilot tests.

Testing by MS/MS is not yet available in kit form. The details of the methodology utilized by the NENSP have been published. 7

The principles upon which the methodology is based will be reviewed briefly.

Normal human functioning requires the conversion of many molecules into other molecules to release and store energy, form structural components, and eliminate unneeded byproducts from the body. Feedback mechanisms provide strong homeostatic pressures on the entire system, and so the levels of biochemicals in the blood tend to remain within predictable ranges in healthy individuals. Metabolic screening has always taken advantage of the fact that metabolic diseases often express themselves through imbalances in the normal levels of blood components and the particular molecules or groups of molecules that are out of range give strong clues to the underlying disorder. Tandem mass spectrometry has earned a lot of excitement in recent years in the newborn screening field because of its power to quickly measure levels of multiple blood components.

The NENSP uses tandem mass spectrometers to measure levels of 29 blood components that provide markers for 23 disorders. Prior to our implementation of tandem mass spectrometry, three of these 23 disorders (PKU, MSUD, and homocystinuria) were screened using bacterial inhibition assays.

Disks punched from the baby’s filter paper blood sample are placed into microtiter wells, and blood components are eluted using a methanol solution containing deuterium-labeled internal standards. The methanol extract is transferred to a new microtiter well and dried. The dried analytes are butylated by the addition of butanol-HCl and 65 °C incubation. The wells areagain dried and reconstituted with acetonitrile and water. The microtiter plates of reconstituted analytes are placed on an autosampler, which injects 15µL of the sample solution into the MS/MS. A sample is injected every 1.9 minutes and the MS/MS analyzes each sample for up to 29 amino acid and acylcarnitine markers.

When the processed extract is delivered to the MS/MS, the instrument filters the analyte and deuterium-labeled analytes according to their mass per unit charge (m/z). Analytes of the same m/z will pass through a collision cell resulting in fragmentation of the molecule into product ions. The resulting product ions are then counted by the detector. Generally the product ions reflect the relative amount of the analyte of interest. Thus by comparison of the counts of the product ion of the analyte of interest to the counts of the product ion of the deuterium-labeled internal standard (whose concentration is known), the software can calculate the concentration of the analyte in the dried blood spot.

Note that samples from babies whose parents chose not to participate in the pilot testing are only assayed for analytes associated with the mandated disorders. The mandated disorders tested by MS/MS are PKU, MSUD, homocystinuria, and MCAD.

In addition to the MS/MS analysis, samples from babies yielding a positive MS/MS screen for the ‘new’ mandated metabolic disorders (MCAD) are also analyzed for the common mutation associated with this disorder (985A→G) using a DNA amplification assay. Physicians of all babies with positive MS/MS screens are notified of the out-of-range result and the additional DNA information gives early supporting information as to the likelihood that the out-of-range result represents an actual MCAD disorder.

During the first three years of MS/MS screening in Massachusetts, 239,690 babies were screened for MCAD, and more than 99.9% of these babies’ specimens yielded within-normal-range screens. There were 78 babies gleaned from all those screened and eight of the 78 were shown through follow-up diagnostic testing in metabolic specialty clinics to have MCAD.

Likewise, of the 236,654 babies screened for the pilot metabolic panel, over 99.8% yielded within-normal-range screens for all 19 of the pilot disorders. Of the 341 screened positive babies, 17 babies were shown to have one of the disorders targeted by the pilot metabolic screen. The specific disorders diagnosed for these babies are shown in Table 4.

Normal ranges for each marker were initially determined by analyzing the marker distribution in 4,000 babies. Recent studies have

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Confirmed Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARG</td>
<td>1</td>
</tr>
<tr>
<td>ASA</td>
<td>1</td>
</tr>
<tr>
<td>CIT</td>
<td>1</td>
</tr>
<tr>
<td>CPTII</td>
<td>1</td>
</tr>
<tr>
<td>LCHAD</td>
<td>1</td>
</tr>
<tr>
<td>MCC</td>
<td>1</td>
</tr>
<tr>
<td>MMA</td>
<td>1</td>
</tr>
<tr>
<td>PA</td>
<td>2</td>
</tr>
<tr>
<td>SCAD</td>
<td>5</td>
</tr>
<tr>
<td>VLCAD</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4. Disorders detected in the first three years of Pilot Study 2
revealed that the mean levels of some of the markers vary according to the weight and age of the baby at the time the sample was drawn. Weight and age-dependent cutoffs should further improve the specificity of the screen.

The natural history of asymptomatic babies with these disorders, detected through population-based screening, is not well understood. To date, the babies with MCAD have done very well, which is promising because of an earlier report that 25% of clinically presenting MCAD patients die upon initial presentation.6 On the other hand, some of the babies with pilot metabolic disorders have not fared as well, despite timely screening and detection. The NENSP is currently involved in a three-year HRSA-supported study designed to objectively analyze the validity and utility of the application of these new technologies to newborn screening.

CONCLUSION
Newborn blood screening is an example of the application of laboratory science to public health. Newborn screening can prevent or ameliorate mental retardation and early death. Effective expansion of newborn screening is the result of linking new technological capabilities with new understandings of disease and developing medical interventions.

REFERENCES
FOCUS: NEWBORN SCREENING

Continuing Education Questions

FALL 2002

To receive 3.0 contact hours of basic level P.A.C.E.® credit for Focus: Newborn Screening, insert your answers in the appropriate spots on the immediately following page; then complete and mail the form as directed.

NOTE: There may be more answer spaces on the answer sheet than needed. If so, leave them blank. Make sure the number of the answer space you fill matches the number of the question you are answering.

LEARNING OBJECTIVES
Newborn screening: an overview
At the end of the article, the learner will be able to:
1. Identify the ethical issues surrounding newborn screening.
2. Discuss the impact of a lack of nationally adopted standards on newborn screening.
3. Describe the criteria used for incorporation of a test in a newborn screening program.
4. Discuss the impact of technological advances on newborn screening.
5. Explain the principle of tandem-mass spectrometry.
6. Identify the pre- and post-analytical variables related to newborn screen testing.
7. Define the deficiency and discuss the physiologic implications associated with the inherited metabolic diseases presented.
8. Discuss the relative incidence of the 12 inherited disorders described.
9. Compare voluntary and mandatory screening.
10. List the factors that, historically, impeded early diagnosis of inherited metabolic diseases.
11. List the two disorders screened by all U.S. states.
12. List the fifteen states that offer testing (at least MCAD) for fatty acid oxidation disorders.

Newborn screening: new developments in a proven field
13. Discuss the relationship of a test’s clinical use to the assay performance measures.
14. Explain the public health issues pertaining to newborn screening.
15. Describe the newborn screening model used by the New England Newborn Screening Program (NENSP).

16. Describe NENSP’s two-tiered testing approach for cystic fibrosis.
17. Discuss the use of tandem mass spectrometry in the NENSP’s pilot program.
18. List the 20 tests included in NENSP’s expanded pilot program.

NEWBORN SCREENING: AN OVERVIEW

1. Which of the following is an important criterion used to justify including a test as part of a newborn screen?
   a. Parental opinion
   b. Early treatment is available
   c. All testing sites easily adopt analytical method
   d. Disease incidence is at least 1:1,000 live births

2. Who is responsible for payment of treatment in affected infants?
   a. Parents
   b. State
   c. Federal
   d. No standard policy exists and the answers vary between states

3. Explain why states differ in their newborn screening test menus.
   a. Funding and budgetary constraints
   b. Ethnic populations and therefore disease prevalence varies between states
   c. Differences in local values
   d. Each state independently adopts its own public health policies
   e. All of the above

4. When is the ideal time to collect blood for newborn screening tests?
   a. Before 24-hours of age
   b. Before discharge from the nursery
   c. Immediately after the infant’s first feeding
   d. Between 48- and 72-hours of age

5. Thus far, which technological advance has had the greatest impact on newborn screening?
   a. Bacterial inhibition assays (Guthrie)
   b. Photometric immunoassays
   c. Tandem mass spectrometry
   d. DNA analysis

Vicki S Freeman PhD, of the Department of Clinical Laboratory Sciences, University of Texas Medical Branch is P.A.C.E.® liaison for the CLS Continuing Education section. She reviews Focus articles, assigns contact hours, and edits learning objectives and test questions.
Direct all continuing education inquiries to Vicki S Freeman PhD at (409) 772-3056, (409) 747-1610 (fax). vfreeman@utmb.edu
6. Which newborn screening test has the highest incidence in Yupik Eskimos?
   a. Congenital adrenal hyperplasia
   b. Congenital hypothyroidism
   c. PKU
   d. Sickle cell disease

7. How many states currently offer FOD (including MCAD) testing by MS/MS?
   a. Six
   b. Seven
   c. Fifteen
   d. Thirty-two

8. Which disorder results from a defect in branched-chain amino acid metabolism?
   a. Biotinidase deficiency
   b. Galactosemia
   c. Homocystinuria
   d. MSUD

9. A newborn exhibits the following clinical symptoms: lethargy, metabolic acidosis, marked hyponatremia, hyperkalemia, and hypovolemia. What is the most likely inherited metabolic disorder?
   a. Biotinidase deficiency
   b. Congenital adrenal hyperplasia
   c. PKU
   d. MSUD

10. The incidence of sickle cell disease is estimated as 1:400 in the African American population. Would an infant from this ethnic group born in South Dakota be tested for this disease as part of the newborn screen?
    a. No
    b. Yes
    c. Yes, but no other hemoglobinopathy
    d. Only if the parents requested the testing

11. What is the fundamental difference between mandatory and voluntary screening?
    a. In a mandatory program, informed consent is required.
    b. In a voluntary program, parents receive the testing results.
    c. In a mandatory program, all newborns are tested without prior consent.
    d. In a voluntary program, parents must request the testing and agree to pay for these services.

12. While it is desirable for any laboratory test to be 100% sensitive and specific (but not realistic), why is extraordinarily high specificity desired for newborn screening tests?
    a. In order to minimize issues dealing with follow-up testing and false-positive results
    b. Prevalence in the general population impacts the likelihood of a true positive
    c. The number of infants tested adversely impacts the specificity
    d. In order to minimize pre-analytical variables

13. Which administrative public health model is favored by Massachusetts?
    a. Separation between each phase of the program which are housed in the most appropriate department or agency.
    b. All operative responsibilities should be housed under one administrative body.
    c. Pre-analytical and analytical phases should be administered under one department or agency with both reporting to a single follow-up group having overall administrative power and responsibility.
    d. Except for the analytical phase, the infant’s medical home should coordinate all other aspects of newborn screening.

14. Describe the two-tier approach to NENSP’s testing for cystic fibrosis.
    a. Tandem mass spectrometry constitutes the initial screen followed by IRT (immunoreactive trypsinogen testing).
    b. Bacterial inhibition is used for initial screening. All positive tests are confirmed by fluoroimmunoassay for trypsinogen elevation.
    c. Those samples from an IRT (immunoreactive trypsinogen testing) run yielding the highest 5% of IRT results prompt DNA testing (PCR) to detect 27 prevalent gene mutations.
    d. Those samples from an IRT (immunoreactive trypsinogen testing) run yielding the highest 5% IRT results prompt sweat testing. Those infants having a positive or inconclusive sweat test result prompt DNA testing.

15. Tandem mass spectrometry allows measurement of 20 to 30 blood components. What is the basis of identification?
    a. Absorbance energy spectra
    b. Comparison to an internal standard of known concentration measured by isotope-dilution techniques
    c. Initial derivatization of classes of compounds, separated by their respective physical properties (charge, solubility, volatility, etc.) are quantified by comparison to a known concentration of the isolated substance
    d. Mass-to-charge ratio of fragmented ions quantified when compared to the concentration of known standards
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(08) CREDIT CARD # ____________________________ TYPE (CIRCLE)    AE     MC     VIS       EXP. DATE __________

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- [ ] I have previously participated in Focus
- [ ] I would like information on other continuing education sources

Answers

Circle correct answer (questions are on previous two pages).

1. a b c d e 8. a b c d e 15. a b c d e 22. a b c d e
2. a b c d e 9. a b c d e 16. a b c d e 23. a b c d e
3. a b c d e 10. a b c d e 17. a b c d e 24. a b c d e
4. a b c d e 11. a b c d e 18. a b c d e 25. a b c d e
5. a b c d e 12. a b c d e 19. a b c d e 26. a b c d e
6. a b c d e 13. a b c d e 20. a b c d e 27. a b c d e
7. a b c d e 14. a b c d e 21. a b c d e 28. a b c d e

2. Specialty: (a) biochemistry/urinalysis (b) microbiology (c) lab administration (d) hematology/hemostasis (e) education (f) immunology (g) immunohematology

3. Workplace: (a) hospital over 500 beds (b) hospital 200–499 beds (c) hospital 100–199 beds (d) hospital under 100 beds (e) private lab (f) community blood bank (g) group practice (h) private physician (i) clinic (j) other

4. Salary range: (a) under $10,000 (b) $10,000 to $20,000 (c) $20,000 to $30,000 (d) $30,000 to $40,000 (e) over $40,000

5. Did these articles achieve their stated objectives? (a) yes  (b) no

6. How much of these articles can you apply in practice? (a) all (b) some (c) very little (d) none

7. Employment status: (a) full time (b) part time (c) student (d) not employed (e) retired

8. How long did it take you to complete both the reading and the quiz? __________ minutes

9. What subjects would you like to see addressed in future Focus articles?

10-CE Questions 10/10/02, 11:34 AM
TRENDS AND TECHNOLOGY

Trends and Technology: Fall 2002

MARY JANE GORE

Trends and Technology welcomes releases and information about new products, services, Web sites, trends, and upcoming events. If your company has a Web site that you would like for us to review, please send us news for our Online section, or tell us about sites that would interest clinical laboratory scientists. These sites, as well as the new product information, are offered for reader information only. We cannot vouch for them and their presence here does not constitute an endorsement by CLS or ASCLS.

FDA NEWS

Quantimetrix has received FDA clearance for its Lipoprint® System platform. This system is intended for use with the previously approved Lipoprint LDL gels to determine low-density lipoproteins in patient specimens. The Lipoprint LDL is the only FDA-approved test system for LDL subfraction testing. Contact Monica Jacobs at (310) 536-0006, x 154.

NEW PRODUCTS

An upgraded and versatile video-camera-based image analysis system has been introduced by Optomax of Hollis, NH. The Optomax Sorcerer Image Analysis system features a high-resolution 1300 x 1030 pixels CCD camera that is totally compatible with a compact Petri viewer or an optical microscope, and can analyze objects as small as 1 mm. Compliant with FDA rules for R&D quality and quality assurance testing, the system is suitable for colony counting and sizing, unscheduled DNA synthesis, Ames testing, Elispot assay, MIC assays, and other life sciences applications. Contact Michael J Huns at (603) 465-3385.

Optomax Sorcerer image analysis system

To continue responding to laboratories’ increasing need for productivity and precision, Dade Behring has introduced the StreamLAB™ Analytical Workcell, designed for high-volume laboratories. The StreamLAB system efficiently integrates fully-automated instruments within a single laboratory, to optimize their combined capabilities. This is achieved within an open system that can be configured and reconfigured to meet individual laboratories’ requirements. Operating like a single workstation from a touch screen, the StreamLAB workcell is compatible with Dade Behring Dimension® RxL systems. Contact Melissa Ziriakus at (847) 236-7038.

Olympus America’s Diagnostic Systems Group has a new operating model that uses standardization to increase productivity and decrease operating costs. The standardization covers chemistry immuno testing from 400 to 6600 tests per hour, whether they use the Olympus AU400, AU640, AU2700, or AU5400 as their primary analyzer or dedicated specialty unit. The standardization model encompasses similar-user interfaces; standardized results and reference ranges; the same calibrations and controls; as well as the same reagents, consumables, and parts. This standardization also reduces training time and expedites cross training. In addition, Olympus has a new solutions-based service to aid laboratory administrators in identifying the most appropriate configuration of instrumentation for greater productivity and faster return on investment for their departments. For more on the standardization model or the workflow simulation Olympus LabModel™, contact Timothy Votapka at (631) 756-7160.

Olympus Diagnostic Systems Group

Watson-Marlow Bredel, which manufactures peristaltic pumps, has introduced its new 400FD/A pump for sample preparation and high throughput screening equipment. These pumps offer the advantages of continuous flow capability and simple plumbing and eliminate the need for valves. Contact Diane Reid at (617) 367-0100, EXT. 105.

Tecan Systems has launched a next-generation compact, high-performance pump. This new pump is designed for automat-
ing precision liquid handling applications in the 5 µL to 5 mL range and is ideal for the clinical setting. Contact Johan Christiaanse at (408) 953-3100.

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**TRENDS AND TECHNOLOGY**

Tecan Systems pump

SAFETY NEWS

Confusion in identifying patients, miscommunication among caregivers, wrong-site surgery, infusion pumps, medication mix-ups, and clinical alarm systems will be the focus of the National Patient Safety Goals for 2003 set by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). For each of the National Patient Safety Goals, there are clear, evidence-based recommendations to help healthcare organizations reduce specific types of healthcare errors. Beginning January 1, 2003, the more than 17,000 JCAHO-accredited healthcare organizations that provide care relevant to the goals will be evaluated for compliance with the recommendations or implementation of acceptable alternatives. The Joint Commission expects to issue National Patient Safety Goals and Recommendations each year. For further information contact Charlene Hill at (630) 792-5175.

Abbott Laboratories today announced the launch of a new needle stick prevention Web site, http://www.abbottnps.com. This site provides healthcare providers with information about current needle stick prevention legislation, both on a federal and state level, as well as educational information about preventing needle stick injuries. According to the Centers for Disease Control and Prevention (CDC), approximately 600,000 to 800,000 injuries from needle sticks occur every year. Visit the web site to answer your questions about preventing needle sticks.

ONLINE

The CDC continues to update and enhance its Web site offering bioterrorism information and resources for health professionals and the public. Found at http://www.bt.cdc.gov/, the site’s laboratory information page has Power Point slide show overviews on bioterrorism, such as specific roles of different types of laboratories in the event of an attack and how laboratories should respond; a second overview is on “Agents of Bioterrorism,” which describes specific tests Level A microbiology laboratories should perform to rule out a suspected organism or refer to another laboratory (this is both a slide show and a PDF file online). Other features on the laboratory page include articles by type of agent (Bacillus anthracis, Brucella, and spot tests of selected organisms; biosafety; packaging protocols for specimens; transfer regulation; training videos, such as “Smallpox: What Every Clinician Should Know”; and a PDF on the Laboratory Response Network for Bioterrorism.

One medical library noted the value of the training Webcasts with this recommendation: “One particularly useful link available from the CDC Bioterrorism web site is the Training link. It is accessible from the Web site’s front page, and provides access via your computer’s media player to archived Webcasts.” The CDC considers this the official federal site for up-to-date and accurate information about managing potential health threats.

Marketlabinc.com, based in Grand Rapids, Michigan, specializes in finding useful products for industrial and clinical laboratories. The company has a team that researches a variety of resources for products that meet the special needs of the laboratory. They also listen to customer opinions to get new ideas about which products to offer on this online distribution site. The company prizes its high service levels for clients and has operators available to assist clients. They offer a free, 30-day trial on most every item they carry and offer hassle-free returns. Contact Marketlab at (800) 237-3604 or fax to (616) 656-2475 (Michigan) or visit the site online at marketlabinc.com.

A new virtual classroom is now available to laboratory with the launch of KnowIT™ (Knowledge-building Interactive Technology), for use by Dade Behring customers with MicroScan® LabPro Information Manager. KnowIT is on-line courseware that provides an interactive learning on-site, directly within the laboratory, by logging on to www.dadebehring.com. Please visit the site for more information.

ARRANGEMENTS

Cholestech Corporation has signed an agreement with Abbott Laboratories for the global distribution of the Cholestech GDX™ System for the determination of glycated hemoglobin. Under terms of the agreement, Abbott will offer the Cholestech GDX system to physician offices and hospital-based customers both domestically and internationally. The company also offers the Cholestech LDX® System to these customers. When combined with the Cholestech GDX System, the LDX system helps offer a full complement of products for immediate risk assessment and therapeutic monitoring of heart disease and diabetes. For more information, visit www.cholestech.com.

Broadlane Inc, which delivers supply cost management services to the healthcare industry, has awarded a five-year agreement to bioMérieux to provide blood culture equipment and instrumentation to Broadlane’s customers. Contact Bob Bokerman at (314) 506-8090.

HealthTrust Purchasing Group has awarded Roche Diagnostics Corp. a multisource contract to supply blood gas and electrolyte products to HPG member facilities and affiliates. The three-year-contract covers the full line of Roche blood gas and electrolyte systems. Contact Michael Boner at (317) 521-2318.
The index to volume 15 of Clinical Laboratory Science is composed of two parts: an Author Index and a Subject Index (p 254).

Abbreviations Used:
AB = Abstract
CP = Clinical Practice
ED = Editorial
FE = Feature
FO = Focus
RP = Report
RS = Research
TT = Trends and Technology
WB = Washington Beat

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