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Focus: Gene-based Diagnostics

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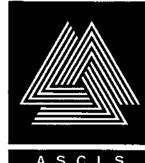

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State Licensure Update: Giving Voice to the Value and Vision

KATHY HANSEN, DON LAVANTY

A session entitled "State Licensure and Legislative Issues" is a perennial event on the ASCLS Annual Meeting agenda. It always draws impressive attendance as various state societies share their experiences in learning about the licensure process, building coalitions with other laboratorians, drafting bill language, finding their way through the state legislature, and responding to those who oppose licensure.

This year's panel of speakers focused mainly on sources of opposition that have been encountered by various state licensure committees, and strategies for responding to that opposition. There was a short introductory portion on licensure basics that can be found in more detail on the ASCLS Web page.

Clinical laboratory science practitioners are licensed in eleven states and Puerto Rico. According to information gathered by the ASCLS Government Affairs Committee, approximately twenty other states are in some phase of licensure activity, from preliminary discussions on through having bills submitted in their state legislatures. Many laboratorians feel passionately about the advantages of licensure to the patients they serve and to themselves as professionals:

- Protect the public health and safety; assure quality of laboratory services,
- Create a mechanism to identify, locate, and mobilize practitioners in the event of a bioterrorism or other public safety threat, and
- Protect the scope of practice of laboratory professionals.

Opposition to proposed state licensure laws has historically come from pathology professional organizations, from state hospital associations, and sometimes from other organizations representing laboratorians, such as the American Association of Bioanalysts (AAB).

However, the American Society for Clinical Pathology (ASCP) issued a policy statement in June 2005, which is solidly in favor of personnel licensure, and puts the organization on the opposite side of the issue from the College of American Pathologists (CAP). The ASCP Policy reads as follows:

"Because the important work performed by laboratory professionals affects the health, safety, and welfare of the public, the American Society for Clinical Pathology (ASCP) believes that states should license laboratory personnel. Licensure legislation would ensure that laboratory personnel possess appropriate academic and clinical training, pass competency-based examinations conducted by an approved national certifying organization, and participate in continuing education programs."[1]

Although the regulations of the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) have some standards for academic and clinical training, these are considered the minimum level by many in the laboratory profession. CLIA is silent on certification examinations and does not have specific requirements for continuing education.

In preparation for developing its new policy statement paper, ASCP surveyed its members about their opinions on licensure with interesting results:

"Over 68,000 ASCP members, including 7,766 pathologists, were asked to participate in an Internet-based survey. More than 10,000 members, including 544 pathologists, completed the survey. The response rate among the overall membership was 15 percent and slightly more than 7 percent for pathologists. To our knowledge, it is the most comprehensive and detailed survey on the issue of laboratory personnel licensure.

Support for licensure was clear and unequivocal; approximately 72 percent of all respondents indicated support while only 18 percent indicated opposition (10 percent expressed no opinion). The ratio of support to opposition was approximately 4:1. Support for licensure among pathologists was more than 2:1; 62 percent supported licensure while 26 percent were opposed (12 percent expressed no opinion)."[1]

In contrast, CAP has testified in opposition to the licensure bill introduced in Massachusetts and its position is also held by the Illinois Society of Pathology, which opposes the proposed bill in Illinois. Their positions have been set forth on their Web site:

- No established link between state licensure of clinical laboratory personnel and discernable improvements in laboratory quality.

- Personnel qualifications are arbitrary, excessively stringent, and not commensurate with the demands of the positions for which licensure is contemplated.
- Licensure would substantially limit entry into the clinical laboratory workforce and exacerbate personnel shortages.
- Legislation would limit the authority of pathologists to select, assign, and qualify personnel.
- Changes in technology require less expertise.[2]

There are data from the federal agency, the Health Resources and Services Administration (HRSA), that show no significant difference between licensure and non-licensure states in shortages of personnel, and also no significant differences in salaries. The CLIA program has published numerous reports linking failures in quality to untrained personnel. ASCLS emphatically disagrees with the statements that personnel qualifications are excessive and that changes in technology require less expertise. New complex laboratory tests are constantly being introduced. We also believe that, in actual practice, selecting and assigning personnel is the province of the laboratory manager and not the pathologist.

State hospital associations oppose licensure because of personnel shortages and because they fear it will increase salaries. There are no data to confirm either of these fears. The concerns raised by AAB include opposition to the baccalaureate degree as the requirement for career entry for a clinical laboratory scientist, and fears that some people currently working in these roles would lose their jobs. All licensure bills that have been introduced include a 'grandfather' provision to ensure that no current practitioner would lose their job. They do set standards going forward from the time of implementation.

Licensure efforts take a number of years and are not easy. We are in this for the long haul—if we don't convey the value of the laboratory and our visions for excellence and safety in our very important part of patient care, no one will do it. Will you commit to lending your voice and your energy when called upon? Will you spread the word among your colleagues and help them to become committed as well?

REFERENCES

1. The American Society for Clinical Pathology Policy Statement. State Licensure of Laboratory Personnel (Policy Number 05-02). Lab Med;Sept 2005.
2. Statline. CAP, Massachusetts pathologists block licensure legislation. Federal and State News Briefs 2004;20(16). www.cap.org/apps/docs/statline/stat080404.html. Accessed August 16, 2005

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Transitions

Some decisions are not all that difficult. After a full and complex life at the University of Iowa, Marian and Ivan Schwabbauer have decided to retire. While at first glance that doesn't seem to impinge upon your life, a second glance at the masthead of Clinical Laboratory Science will bring a mixed reality home. The Schwabbauers have served first as Editor-in-Chief and then, most recently, as the Editorial Office. From improving turn around time to modernizing the process and making it electronically based, they have brought the journal process into the twenty-first century with style and graciousness. Often a person's effect on others is not known. So, here are just a few of the people they have helped and taught to be better editors, better reviewers, and better writers. Susan Leclair, Bunny Rodak, Kathy Doig, George Fritsma, Carol McCoy, Cheryl Caskey, and all of the guest editors. The list runs into the hundreds.

They have agreed to help the new editorial office staff adjust to their new responsibilities with the next issue of the journal and will be enjoying their new life by the end of this calendar year. While the editors are pleased that the Schwabbauers will be able to travel and enjoy their lives, we are intimately aware of our loss. Thank you Marian and Ivan for more than you know.

Susan Leclair, Clin Lab Sci Editor-in-chief

Thanks for the Memories

As William Bridges points out in his seminal book on transitions, there must be an ending before there can be a new beginning.[1] It is with a bittersweet mixture of sadness and anticipation that we have decided to completely retire as of this issue of *Clinical Laboratory Science.* Not only is this the last issue of Volume 18, it is our last issue as the Editorial Office. During the nine plus years that we have been responsible for *Clinical Laboratory Science,* we were privileged to have the opportunity to work with many dedicated and talented authors, reviewers, and editors. For the most part, those years have been very rewarding, and 'Big Fun'.[2] However, we are looking forward to having more time to spend with our family, more freedom to travel, and more energy to devote to our hobbies and volunteer activities, also rewarding and 'Big Fun'.

Margaret LeMay will assume *Clinical Laboratory Science* Editorial Office functions beginning with the first issue of Volume 19. We confident that *Clinical Laboratory Science* will continue to advance under Ms LeMay's capable guidance. We have already begun working with Ms LeMay to ensure a seamless transition. We trust that you will give her the same support and encouragement that you gave us when we took over the editorial office duties.

We wish *Clinical Laboratory Science* and its readers all the best in the future. It's been fun!

Ivan and Marian Schwabbauer
Clinical Laboratory Science Editorial Office

1. Bridges W. Transitions: making sense of life's changes. Reading MA: Addison-Wesley; 1980. 170 p.
2. Joan McCloskey defines 'Big Fun' "as the good feeling we get from being creative, making others feel good, and being good at what we do" in: Creating an environment for success with fun, hope and trouble. J Nurs Adm 1991;21:4:5-6.

The Dialogue and Discussion Section is a forum for editorials, short articles, commentaries, and letters to the editor on clinical laboratory science topics and professional issues of general interest to readers including ASCLS activities and position papers. For more information about submissions to the Dialogue and Discussion section contact: Susan Leclair PhD, Editor-in-Chief, Clinical Laboratory Science Editorial Office, Attn: Dialogue and Discussion, PO Box 5399, Coralville, IA 52241-5399. (319) 351-2922, (319) 351-2927 (fax). cls@ia.net

LETTER TO THE EDITOR

Future Career Paths— Is it Time to Phase Out AS-CLT Programs?

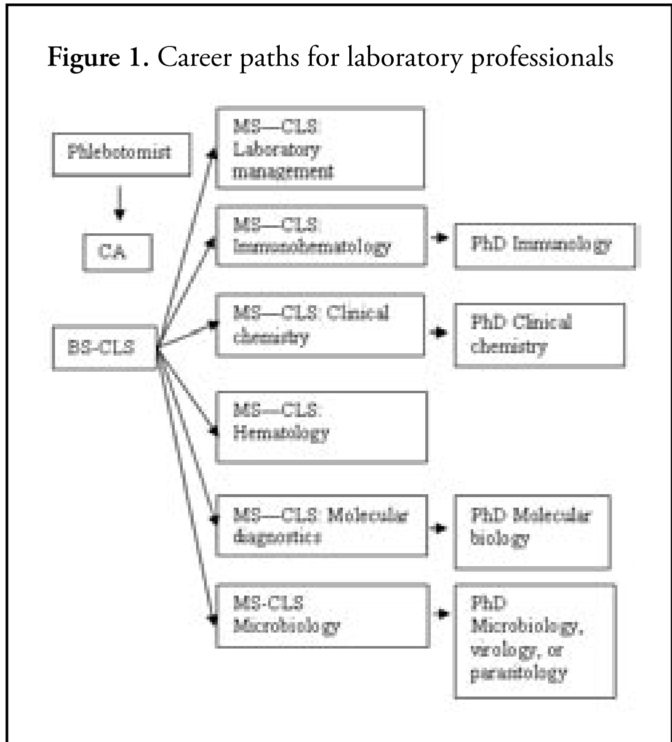
HEIDI A MANNION

In reviewing Dr Kathy Doig's editorial, "The Case for the Professional Doctorate in Clinical Laboratory Science (DCLS)", I agree with her statement that there is a high degree of overlap between the entry-level positions of a clinical laboratory technician (CLT) and a clinical laboratory scientist (CLS) in some facilities which may lead to dissatisfaction and a higher attrition for both. I also agree that the current career paths need to be revised. However, requiring a master's degree (MS) for entry-level or a clinical doctorate (DCLS) is not the solution to the problem. I do not believe there is a market for the DCLS. In addition to the clinical pathologists who are already providing consultations, recent advances in information technology allow physicians and other healthcare professionals access to information on ordering and interpreting laboratory tests.

It is time to consider phasing out the CLT. The addition of new content makes it difficult to adequately cover the required material in the 60 credits necessary for an associate degree. A bachelor's degree should be mandatory for entry-level positions, with phlebotomists and clinical assistants (CA) performing blood collection, waived testing, and appropriate tests at the clinical assistant level, according to standard operating procedures. In order to accommodate new content BS-CLS curricula need to be revised to eliminate coursework that is no longer necessary due to automation and other advances in laboratory science. CLT programs could be converted to either BS-CLS or Clinical Assistant Programs and certifying agencies could revise the CLT/MLT examinations and utilize them for certifying clinical assistants.

In addition to phasing out the CLT, we should consider eliminating the specialist certifications. With the exception of the SBB most specialist certifications are not recognized outside of the clinical laboratory, however an MS or PhD would receive the appropriate recognition. Clinical laboratory scientists who want to become a specialist in clinical practice, teach, or conduct research in a specific area would receive more recognition if they completed an MS, and in some cases a PhD, in the subject area. In the career paths depicted in Figure 1, the master's degree would be in clinical laboratory science with multiple tracks. The same core subjects including research and design, statistical analysis, bioethics, management, and education (15 credits) would be required for all tracks. In addition to the core subjects, courses specific to the track would be required for the degree. Offering the MS-CLS by distance delivery would allow generalists the opportunity to specialize in their area of interest while remaining in the workforce and would reduce the number of faculty needed if only a limited number of colleges offered the program.

Figure 1. Career paths for laboratory professionals



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LETTER TO THE EDITOR

Clinical Doctorate in Laboratory Science

ELLIS M FROHMAN

I found the two articles concerning doctorate level clinical laboratory scientists (CLS) (Doig K, The Case for the Clinical Doctorate in Laboratory Science, Clinical Laboratory Science 2005;18(3):132-6, and Fritsma GA, A Professional Doctorate in Clinical Laboratory Science?—Not so Fast, Clinical Laboratory Science 2005;18(3):137-8), interesting and at the same time troublesome. The pros and cons of doctoral degrees in clinical laboratory science has been the subject of debate for the better part of the past 20 years.

This debate is akin to the chicken vs. the egg argument—which came first? Do we create doctoral CLSs (DCLSs) and have them go forth to find a purpose within the healthcare team or do we establish a purpose and then create DCLSs to fill the void? At this time we seem to have a potential product without a market.

The comparison between the role of the DCLS and the role of the PharmD is weak at best. The PharmD directly interacts with the clinician, nurse, laboratory, and patient in finding the right drug or combination of drugs to achieve a particular outcome. This is more than a consultative role. They are engaged in direct patient care.

The DCLS, as described, is a purely consultative role with the clinician, advising on the best test to order or providing interpretive information pertaining to the test results. Is this not the role of the clinical pathologist? In fact, I believe it is, but unfortunately it is not done well or consistently in many facilities which has been the subject of a number of CAP TODAY editorials.

I find little, if any, economic justification for the DCLS. In today's healthcare market of rising costs and decreasing reimbursements, there is little support for hiring costly personnel to provide non-billable services whose value has not been established. The value of the DCLS and the service provided must be determined by the user, who, to my knowledge, has shown little interest in using consultative services currently provided by the clinical pathologist.

The idea of a DCLS is a creation of the laboratory industry not the customer/physician. Physicians order many tests from the laboratory but rarely invite laboratory personnel/pathologists to assist them in their diagnostic decision making. This may not be ideal but it is reality, therefore what makes any of us think that a having a DCLS on the staff will make any difference?

I firmly believe the laboratory industry should be focusing its efforts toward increasing the market value of the CLSs in their role on the healthcare team. Overall the CLS's compensation has fallen well behind other professionals in healthcare. Personnel shortages are related to low salaries which encourage college students to look elsewhere for careers and newly graduated CLSs to look outside of patient care facilities such as industrial laboratories, marketing, etc. for greater rewards. The proliferation of degrees and certifications in the past 20+ years has not improved the compensation of laboratory professionals.

To make up for staffing shortages, clinical laboratories have installed significant levels of automation in the largest to the smallest facilities and consolidated with other laboratories or out sourced their work to commercial reference laboratories. This further depresses salaries; in some laboratories it reduces the need for CLS skill level practitioners, and discourages entry into the field. Can hospitals provide safe, quality healthcare without a professionally staffed clinical laboratory? If the answer is no, then it is high time to make the healthcare industry aware of the value of the CLS and what it will mean to patient care if these dedicated individuals are too few to provide the services necessary.

We are on the threshold of another explosion in testing methods and systems that will move from research and specialty laboratories to routine testing in the clinical laboratory. This testing will be more complex and initially less automated than current test methods. We will see even greater emphasis on faster turn around times to replace or supplement current processes such as culturing and batch testing. We will need skilled practitioners to manage, perform, and interpret the results of these new processes.

ASCLS, CLMA, etc. should be consumed with reversing today's downward spiral in which CLS practitioners find themselves and not spend time creating (or debating) a product (DCLS) for which there is no market.

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The Clinical Laboratory Practitioner

DAVID FOWLER, TINA MARTIN, LIBBY SPENCE

OBJECTIVE: This study was designed to investigate potential areas of practice for the clinical laboratory scientist (CLS) and to propose a graduate curriculum to prepare the practitioner for an advanced level of practice.

DESIGN: Meta-analysis of PharmD, physician assistant, physical therapy, and nurse practitioner curricula focusing on academic and clinical advanced practice was used to develop an educational model and curriculum for a professional doctorate in clinical laboratory science (CLS).

MAIN OUTCOME MEASURE: 1) New educational model for CLS advanced practice; 2) A proposed curriculum for a Doctorate of Clinical Laboratory Science degree.

RESULTS: A new curriculum model was adapted from established healthcare educational models.

CONCLUSION: Although there is a need for a baccalaureate degree in CLS there is also a role for expanded education and responsibilities for CLS practitioners. The CLS Advanced Practitioner design focuses on moving students from the baccalaureate level to the doctoral level and prepares the individual to become an integral part of the healthcare team.

ABBREVIATIONS: CLP = clinical laboratory practitioner; CLS = clinical laboratory science/scientist; IOM = Institute of Medicine; PharmD = Doctor of Pharmacy.

INDEX TERMS: advanced practice; clinical laboratory science; curriculum; laboratory personnel; professional doctorate.

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At this time, a BS degree is the entry-level for the CLS. However, educators have gained interest in pursing development of a graduate curriculum for those students who wish to advance their scope of practice.[1,2] It is the intent of the authors to provide evidence that the development of an expanded role for the CLS could provide economic benefits to healthcare and promote patient safety. Clinical laboratory services provide approximately 60% to 70% of the objective information used in clinical decision-making.[3] In 1999, the Institute of Medicine (IOM) reported that an estimated 98,000 Americans die each year from preventable errors.[4] Although the report does not focus on laboratory errors as a major problem, it would appear that any efforts to promote patient safety would be advantageous. The American Society for Clinical Laboratory Science (ASCLS) supports IOM's recommendation of healthcare professional collaboration and promotion of effective team functioning to raise awareness in preventing laboratory errors.[4,5] Through an interdisciplinary team approach, the clinical laboratory practitioner (CLP) would positively impact patient outcomes and provide cost savings to the healthcare system by providing valuable and reliable clinical based knowledge regarding laboratory testing that fosters accurate and timely diagnoses. This further supports the IOM's report that recommended improved access to accurate and timely information as a way to prevent errors and improve patient safety.[4] The advanced practice CLP may increase efficiency, facilitate patient management outcomes, and improve access to accurate laboratory information by participating in patient care activities. CLSs have extensive knowledge regarding laboratory tests and data and, with advanced training, may help clinicians choose appropriate laboratory studies based on the physiological clinical situation while maximizing clinical benefits and reducing the number of negative outcomes. The impact of positive and cost-effective benefits will need to be established through outcomes research data to determine the best role for the CLP in disease management.

Some allied health programs, including physical therapy and occupational therapy, now require a master's prepared entry-level. Audiology is transitioning toward a doctorate level of entry for practice. Pharmacists now require a PharmD degree as entry level. Several studies have examined the effectiveness of a PharmD on patient outcomes.[6-10] Following the direction of the PharmD education model, we propose a graduate level curriculum for clinical laboratory personnel. To prepare for the CLP's expanded role, the curriculum is designed to require students to complete a professional doctoral level degree. In addition, a certification examination and state licensure must be developed and implemented.

MATERIALS AND METHODS

The educational model was developed after reviewing several PharmD, physician assistant, physical therapy, and nurse practitioner programs. The PharmD programs offered a curriculum that appeared to follow what we believed our course expectations for the CLP should be. We, therefore, structured our curriculum using the PharmD model as a guide. The PharmD curriculum focuses on the importance of academic and clinical background necessary for advanced practice.

The nurse education model was used for the educational design of the proposed program. The nurse education model consists of varying educational opportunities at a particular level. Students are given the option to choose among various education opportunities to obtain a nursing degree at the associate degree or baccalaureate level. At the masters of nursing level, the RN has the preference of selecting the nurse practitioner, educator, nurse manager, or nurse executive role and course of study. Within the nurse practitioner focus, the student may choose among neonatal, pediatric, family, or acute care. The proposed CLP program is designed to offer similar educational tracks. The proposed entry-level clinical laboratory doctorate is a three-year post-baccalaureate degree that prepares students for advanced clinical practice. The knowledge and skills obtained from the baccalaureate degree in CLS are intended to serve as prerequisites.

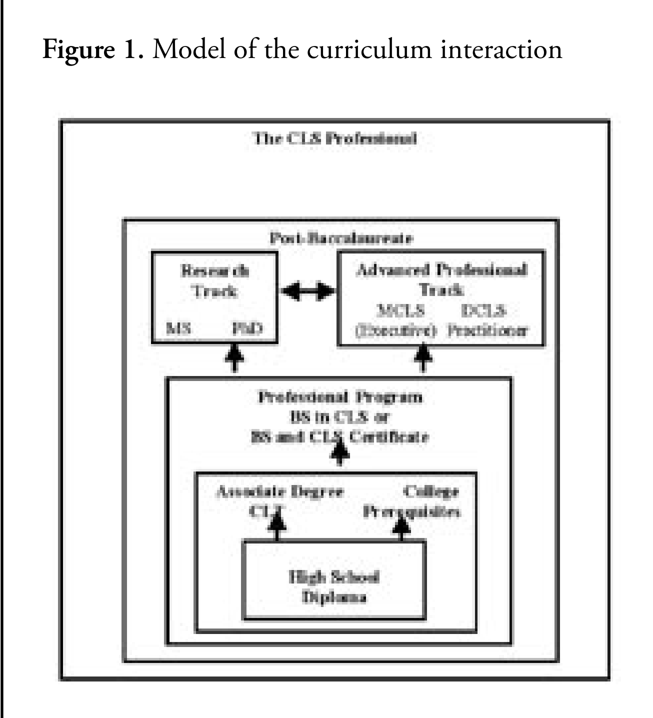


Figure 1. Model of the curriculum interaction

RESULTS

The education and curriculum models utilize a conceptual approach to identify essential learning opportunities for the CLP. Figure 1 describes the educational model of progression for the clinical laboratory professional. Utilizing the knowledge gained in prerequisites, we began to identify areas of study and clinical experiences that are essential to the development of a competent practitioner. Table 1 demonstrates a core educational base consisting of basic, science, and clinical courses, which develops as the student progresses throughout the program. Courses are designed to build upon one another and the student is expected to incorporate knowledge gained into future courses and clinical experiences. Courses in communication and health assessment help the student acquire interpersonal skills necessary for effective communication with patients as well as members of the healthcare team. The student will be able to monitor and evaluate efficacy of laboratory testing by taking patient health histories, observing patients, performing limited physical assessments, determining laboratory tests to order, and performing any appropriate point of care testing.

Advanced courses in the sciences and clinical laboratory provide background to assist the student with diagnostic interpretation and disease management. The clinical curriculum is intended to apply didactic content into the patient care setting and promote critical thinking. Discussions enhanced by seminar courses provide for a continuous sharing of experiences and foster critical-thinking. Students have an opportunity to take up to nine (9) elective hours of coursework to explore their personal interests and varied clinical laboratory opportunities.

Table 1. Curriculum for the Doctorate of Clinical Laboratory Science

Semester	Course Title	Credits
1st year		
Fall	Pathophysiology	4
	Legal Ethics	3
	Communications	2
		9
Spring	Pharmacology	3
	Research Methods—Statistics	3
		6
Summer	Health Assessment	2
	Health Policy	3
		5
2nd year		
Fall	Pathologic Concepts for Clinical Decision Making I	3
	Hematopathology and Flow	3
	Cytometry Issues in CLS I	1
	Elective	3
		7 – 10
Spring	Molecular Diagnostics	
	Pathologic Concepts for	3
	Clinical Decision Making II	3
	Issues in CLS II	1
	Elective	3
		7 – 10
Summer	Point of Care	2
	Elective	3
		2-5
3rd year		
Fall	Clinical Laboratory Diagnosis I	4
	Issues in CLS III	1
	Clinical project	1
		6
Spring	Clinical Laboratory Diagnosis II	4
	Issues in CLS IV	1
	Clinical project	1
		6
Total hours		**48 – 57**

DISCUSSION

The CLP can be a valuable member of the healthcare team. After completing the Doctorate of Clinical Laboratory Sciences, the practitioner collaborates with physicians and other healthcare providers in the diagnosis and treatment of patients by ordering, performing, correlating, and interpreting laboratory tests as well as monitoring patient outcomes. The initial practice area will most likely be hospital-based. As stated earlier, 60% to70% of all clinical decisions are made by laboratory services.[3] The CLP is in a unique position to improve patient outcomes while developing and strengthening collaborative relationships among laboratory services and other healthcare providers. The CLP could be responsible for all testing and interpretation along with point of care testing to improve access to accurate and timely diagnostic information while serving as a liaison between nursing units, healthcare providers, and the laboratory to ensure quality and cost-effective laboratory services. We recognize obstacles may be encountered while implementing this CLP model. However, one feasible way of easing transition may be utilizing the CLP in point of care testing. This may strengthen multidisciplinary relationships and provide a portal of entry into the healthcare setting, above and beyond the laboratory. Careful deliberation is necessary to establish the most suitable entry level for the CLP. Routes of entry for the position include an independent consultant, practitioner, or clinical pathology assistant.

Presently, the only educational options in the CLS post baccalaureate are along the research track and include a master's or PhD. The CLS professional education model offers an advanced professional track at a master's level for a management or executive focus or doctoral level for the CLP role (Figure 1). The curriculum model incorporates courses in the CLS practitioner program (Table 1). It is composed of basic, science, and clinical courses. Professional skills are attained from basic and clinical courses. The cognitive skills are developed from basic, science, and clinical components. Assessment skills are primarily acquired from science and clinical courses. Diagnostic skills are acquired from the culmination of all coursework (Figure 2).

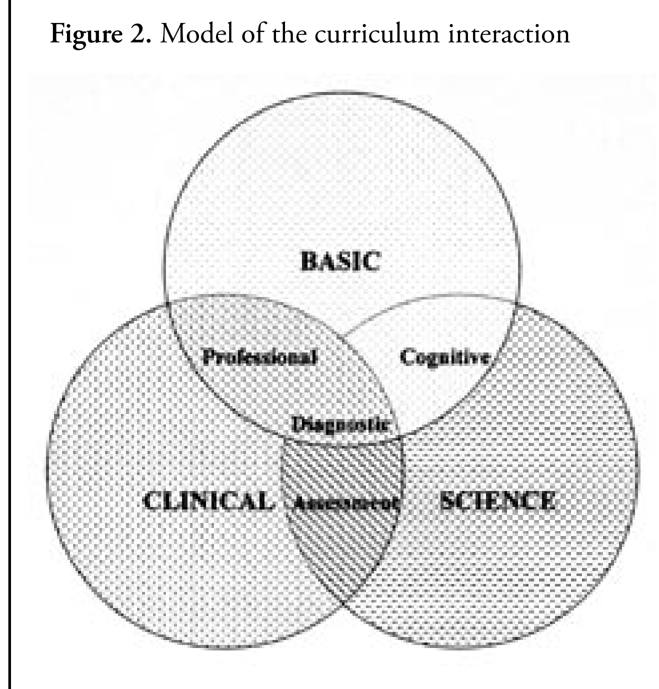
We acknowledge that there is still a need for a baccalaureate degree in CLS but an advanced degree option would allow those who wish to advance their education beyond the baccalaureate degree and enhance career opportunities.

CONCLUSION

The American Medical Association recognizes that laboratory testing plays an important role in detection, diagnosis, and treatment of many diseases.[11] The proposed CLS educational model is designed to offer an alternative course of educational study for the CLS. The CLS practitioner design focuses on moving students from the baccalaureate level to doctoral level and prepares the individual to fill a particular role as a part of the healthcare team. The CLP can be an integral part of the healthcare team and has the opportunity to implement supportive services at the healthcare delivery level under the direction of a collaborating physician to decrease fragmentation of care, decrease hospital stay, reduce costs, and promote patient safety. Educational preparation and certification will prepare the CLP for expanded responsibilities and curricula must be designed to meet these goals.

After determining the appropriate method of entry of the CLP, funding issues must be addressed. Pilot studies are proposed initially in approximately two or three institutions. Possible healthcare settings may include acute care, emergency department, primary care, or family practice. After obtaining results of these pilot studies, we will have a better understanding of the role expectations and scope of practice and determine the most efficient way to implement the educational model. A potential barrier may be in the implementation of the professional position. Education regarding role definition and scope of practice will be essential for smooth transition into the patient care setting. Future projects will most likely be needed to help define role expectations.

Figure 2. Model of the curriculum interaction



Issues that must be addressed once pilot studies are complete include specific job description with salary expectations, physician collaboration, certification/licensure, acceptance by other healthcare providers, cost-benefit analysis, professional liability, and healthcare reimbursement. In today's healthcare setting, cost justification and healthcare reimbursement regulations are crucial. The most probable obstacle will most likely be healthcare reimbursement. New healthcare policies will need to be developed through appropriate governmental agencies. Data regarding patient clinical outcomes utilizing the advanced practice CLP will be essential to help support the new role.

REFERENCES

1. Beck SJ, Doig K. An entry-level MS degree in clinical laboratory sciences: is it time? Clin Lab Sci 2002;15(3):167-76.
2. Fenn JP, Knight JA. The value of graduate education in clinical laboratory science. Lab Med 1995;26(8):537-41.
3. Forsman RW. Why is the laboratory an afterthought for managed care organizations? Clin Chem 1996;42(5):813-6.
4. Institute of Medicine. To err is human: building a safer healthcare system 1999. National Academy Press. Washington DC.
5. American Society for Clinical Laboratory Science Position Paper. Medical errors and patient safety 2001. Available at http://www.ascls.org. Accessed October 8, 2002.
6. Kaushal R, Bates DW. The clinical pharmacist's role in preventing adverse drug events. Available at http://www.ahcpr.gov. Accessed October 8, 2002.
7. Beney J, Bero LA, Bond C. Expanding the roles of outpatient pharmacists: effects on health services utilization, costs, and patient outcomes. In: The Cochrane Library, Issue 2, 2000 (Oxford).
8. Leape LL, Cullen DJ, Clapp MD, and others. Pharmacist participation on physician rounds and adverse drug events in the intensive care unit. JAMA 1999;281(3):267-70.
9. Gattis WA, Hasselblad V, Whellan DJ, O'Connor CM. Reduction in heart failure events by the addition of a clinical pharmacist to the heart failure management team: Results of the pharmacist in heart failure assessment recommendation and monitoring (PHARM) study. Arch Intern Med 1999;159:1939-45.
10. McMullin ST, Hennenfent JA, Ritchie DJ, and others. A prospective, randomized trial to assess the cost impact of pharmacist-initiated interventions. Arch Intern Med 1999;159:2306-9.
11. American Medical Association. Occupational Description-CLS/MLS 2002. Taken from 2002-2003 edition Health Professions: Career and Education Directory. Available at http://www.ama-assn.org. Accessed October 15, 2002.

Effect of Adverse Storage Conditions on Performance of Glucometer Test Strips

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NOTE: This is a student project paper as noted in the text.

OBJECTIVE: A study was conducted to assess the impact of adverse storage environments, i.e., not manufacturer recommended, on the performance of reagent test strips used with a point of care testing (POCT) glucometer to measure whole blood glucose levels.

DESIGN/SETTING: Glucose reagent test strips were placed in open, i.e., uncapped, and closed, i.e., capped vials. These vials were those used by the manufacturer to package and store the reagent test strips. One of each type of vial was placed in the manufacturer-recommended storage environment at room temperature and the adverse environments (incubator, direct light to mimic sunlight exposure, humidity, and refrigerated). The Accu-Chek Easy® glucometer and reagent test strips as well as Accu-Chek Easy high and low glucose control solutions, manufactured by Roche, were used for this study.

MAIN OUTCOME MEASURES: On day-3, day-7, and then once every 7 days, one strip from each vial in each environment was tested with the same glucometer using both a high and a low glucose control. The strip was considered failed for a type of vial and storage environment when either control was out of the reference range on a regular testing day and still out of range when tested the subsequent day. Testing continued up to 50 days.

RESULTS: For the tested environments it was found that, overall, test strip stability lasted longer for closed vials than open vials. For open vials in adverse storage conditions, the refrigerator environment offered the longest stability at 35 to 50 days and direct light and humidity offered the shortest periods of stability at 3 to 14 days.

CONCLUSIONS: The results of this study support the manufacturer's recommendations to store POCT glucose test strips in their original vial, capped, and at room temperature, though refrigeration may offer an alternative storage environment with acceptable stability. As compliance with testing, quality control, and storage instructions is often an issue with POCT, the manufacturers of these systems for blood glucose measurement should design storage systems that allow the patient to store the glucose meter and the reagent strips in the same location. Manufacturers may also need to consider designing storage systems that are more portable, knowing that patients must take the glucose meters and test strips with them when they travel. Roche's Accu-Chek Compact system is an example of such a design. The glucose test strips are incorporated into a drum that is stored in the Accu-Chek meter itself. When a patient performs a fingerstick blood glucose measurement, the drum advances to move a test strip outside the meter. When the test is complete, the test strip is ejected for disposal.[1]

Future studies to clarify the effect of adverse storage conditions, particularly refrigeration, on the integrity of POCT test systems and reagent strips is warranted with currently marketed brands.

ABBREVIATIONS: CLS = clinical laboratory science; CV = coefficient of variation; POCT = point of care testing; SD = standard deviation; µL = microliter.

INDEX TERMS: diabetes; glucometer, glucose meter: point-of-care-testing glucose levels.

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Diabetes affects approximately 6% of the U.S. population, with over 90% of these cases classified as Type 2, or adult onset, diabetes. In addition, it is estimated that there are at least as many persons with pre-diabetes, i.e., blood glucose levels higher than normal but not clinically meeting the criteria for a diagnosis of diabetes.[2] Complications of diabetes constitute a substantial cost in healthcare as well as debilitation to the patient.

FDA approval of POCT glucometers for home use has made it possible for diabetic patients to monitor their own blood glucose levels.[3] The quick turnaround time allows for more rapid intervention via diet or medication for blood glucose values that are above or below desired levels. This has shifted the responsibility for quality control from healthcare personnel to the diabetics themselves. This shift in responsibility to patients has increased the amount of variability present in blood glucose testing, and questions have arisen as to the reliability and accuracy of the values obtained by the patient at home. Inaccurate results can lead to inappropriate, and possibly harmful, adjustment of the patient's medication or diet.[4]

There are a number of factors that can negatively affect the accuracy and precision of glucometer results including user variability, instrument malfunction, and defective reagent strips. Some patients may not necessarily follow all of the manufacturer's instructions for using their glucometers and corresponding test strips. Erroneous results can occur if control solutions are not analyzed routinely to ensure that the instrument and strips are actually working properly, or by not storing the reagent strips according to the manufacturer's instructions. If the drop of blood does not cover the entire testing area, reflectance glucometers can give a falsely low glucose value. These errors may occur as a result of poor patient training provided by non-laboratory personnel or by the patient's negligence in adhering to the proper procedures for in-home testing and quality control.[2,3]

Most glucometer research to date has focused on the actual performance of the test procedure under varying conditions. Testing under conditions of increased humidity or atmospheric pressure has been found to adversely affect the precision and accuracy of glucose results measured by glucometers.[5-9] The reagent strips can be defective due to normal expiration, mishandling, or storing them in environments not recommended by the manufacturer, resulting in inaccurate results. Glucose test reagent strips are supplied by the manufacturer in tightly capped vials, and it is recommended that only one test strip at a time be removed. The cap must then be replaced on the vial immediately and the vial stored at room temperature in a location free of extreme temperature changes. Exposure to light causes discoloration of the test area on the strips, falsely elevating glucose results.[4]

The effect of improper storage of glucose reagent strips used for glucometers has not been well documented in the literature. One study found glucose values falsely elevated after storage of strips in a refrigerator for 24 hours.[10] A study by Gonzales and Kampa using the Accu-Check Easy Glucose Monitor test system, i.e., glucometer and test strips, measured performance of these strips for both capped and uncapped vials under adverse storage conditions which included refrigeration (4 °C to 7 °C), incubation at elevated temperatures (37 °C), exposure to direct sunlight (excessive variable heat), and increased humidity as in a laundry washroom. Deterioration of the test strips as indicated by high and low control results outside their expected ranges, was found to occur the earliest in refrigeration environments for both the uncapped and capped vials. Of the adverse environments tested by Gonzales and Kampa, glucose reagent test strips were most stable in a humid environment.[11]

To supplement the limited research on stability of glucometer test strips under various storage conditions, a replication of the Gonzales and Kampa study with slight modifications was conducted by the researchers. This study used the same POCT glucose monitoring system as in the Gonzales and Kampa study, and served as a senior research project for three clinical laboratory science (CLS) students at East Carolina University (ECU). Faculty served as supervisors and co-researchers for the study. This study, therefore, also offers an example of viable research by CLS undergraduate students.

METHODS

Instrument and reagents

The Accu-Chek Easy Glucometer and Accu-Chek Easy Test Strips by Roche were used in this study to measure glucose in control solutions with low and high glucose concentrations. The test strips contain all the reagents for the glucose oxidase reaction that is initiated when blood is placed on the strip. β-D-glucose in the patient's sample is oxidized and ferricyanide is reduced to ferrocyanide using glucose oxidase as a catalyst. The ferrocyanide reacts with ferric ion to produce a blue color via the Prussian blue indicator, with the intensity of the color being proportional to the glucose concentration in the sample. The color is measured by reflectance photometry.[11] All measurements in this study were carried out according to the manufacturer's instructions.

Specimens and testing

Accu-Chek Easy glucose high and low control solutions were used in this study as the test specimens. One lot number of Accu-Chek Easy Test Strips was used. Two vials of test strips, one open and one closed, were assigned to each storage condition. The storage conditions were room temperature (22 °C to 25 °C) as recommended by the manufacturer, incubator (37 °C), refrigerator (4 °C to 8 °C), light (direct, constant exposure to a 60-watt lamp two inches away), and increased humidity (in a laundry washroom). The adverse environments were chosen to be similar to possible storage environments in a patient's home or on the nursing floors in a hospital. The number of strips in each vial in each storage environment was determined based on the results of the Gonzales and Kampa study as well as to minimize the study costs for the CLS program (Table 1).[11] The temperature in each storage environment except the washroom, was measured for ten consecutive days prior to beginning the study to detect any fluctuations outside the required temperature range. Temperatures were monitored and recorded for each environment during the study.

Table 1. Number of strips stored in each vial in each environment

Storage environment	Closed vial	Open vial
Room temperature	39	26
Refrigerator	26	16
Incubator	28	18
Light	28	22
Humidity	28	26

Glucose concentrations were measured by using one test strip for each control level from each vial in each storage environment. The strips were not allowed to reach room temperature; instead, they were tested at the environment's temperature. Strips in all environments were tested at day-3, day-7, and then once a week on Monday thereafter, according to the manufacturer's procedure.

Methodology evaluation

Accuracy and precision studies were performed on two POCT glucometers owned by the CLS program using the Accu-Chek Easy glucose high and low control solutions. A single glucometer that had both precision and accuracy CVs of less than 5% was used for all testing of reagent strips. Precision studies using gravimetric analysis were performed on the droppers on all four bottles of control solutions (two high controls, two low controls) to verify consistent drop size. All four droppers were found to have CVs of less than 5% with delivery of a drop size of 30 µL.

The manufacturer's acceptable ranges for the high and low glucose control solutions were 202 to 274 mg/dL and 36 to 66 mg/dL, respectively. These ranges were used to determine whether the measured glucose value indicated continued stability of the strip. If the control result for a strip was out of range on a particular day, then another strip was tested on that same day. A third measurement was performed on the following day. The second and third glucose measurements were used to confirm strip failure in a storage environment. When the strips demonstrated failure for duplicate testing on two consecutive days for a vial in a storage environment, that particular environment was noted as having failed for that vial, and testing was discontinued. All testing was stopped at 50 days. The control results for the manufacturer's recommended storage environment, i.e., room temperature in capped vials away from extremes of temperature, were found to be within expected ranges for the duration of the study.

Data analysis

Using Microsoft Excel 2000 software, glucose values (y-axis) were plotted versus the day (x-axis) on which they were obtained for each vial and each environment. The glucose values obtained on the closed vial at room temperature were used as the reference (or comparison) variance value for F-test and mean value for student t-test analyses with alpha set at 0.05 for both statistical analyses.

RESULTS

The day on which each vial in each storage environment failed with regard to a level of control solution is displayed in Table 2. Descriptive statistics by type of vial with significant F-tests and t-tests at $p < 0.05$ noted, are displayed for glucose low control and high control in Tables 3 and 4, respectively. The glucose values obtained by individual testing days by type of vial and level of control solution are displayed in Figures 1 through 4.

Table 2. Reagent strip stability time in days per vial and environment for each control solution

Storage environment	Low control		High control	
	Closed	Open	Closed	Open
Room temperature	>50	14	>50	21
Refrigerator	>50	35	>50	>50
Incubator	28	21	28	14
Light	28	3	35	14
Humidity	>50	3	>50	14

DISCUSSION

With the exception of test strips stored in the refrigerator and tested with the high glucose control solution, Accu-Chek Easy Test Strips stored in open vials deteriorated more rapidly than those in closed vials for each environment and control solution. This result is similar to the findings from the Gonzales and Kampa glucometer reagent strip stability study.[11] The closed vials in the room temperature, refrigerator, and increased humidity environments all remained stable throughout 50 days of testing; whereas in the earlier study, the closed vials in the room

Table 3. Descriptive statistics of glucose values for low control by type of vial

Storage environment	Closed vial			Open vial		
	Mean	SD	Variance	Mean	SD	Variance
Room temperature	59.3	5.2	27.3	68.8*(p 0.023)	3.7	13.7
Refrigerator	54.4	7.9	62.3† (p 0.025)	58.6	11.2	126.6† (p 0.003)
Incubator	65.5* (p 0.001)	4.8	23.4	69.9* (p 0.006)	6.3	39.8
Light	64.4	9.8	95.1† (p 0.017)	81.2	10.6	113.2** (p 0.000)
Humidity	59.8	7.1	51.1	72.8	3.1	9.6† (p 0.004)

* Based on comparison to mean for closed vial at room temperature by student t-test at $p < 0.05$.
† Based on comparison to variance for closed vial at room temperature by F-test at $p < 0.05$.
Expected range of control values was 36 to 66 mg/dL.

Table 4. Descriptive statistics of glucose values for high control by type of vial

Storage environment	Closed vial			Open vial		
	Mean	SD	Variance	Mean	SD	Variance
Room Temperature	256.5	6.9	47.1	271.3	13.7	188.2* (p 0.012)
Refrigerator	247.5† (p 0.005)	7.7	59.1	245.9† (p 0.007)	6	36.1
Incubator	269† (p 0.000)	8.6	74.7	274.3† (p 0.007)	5.1	26.2
Light	231.8† (p 0.312)	38	1445.1	300.3	38.6	1487.1* (p 0.006)
Humidity	257.9	7.2	52.4	274.3	15.6	242.3

* Based on comparison to variance for closed vial at room temperature by F-test at $p < 0.05$.
† Based on comparison to mean for closed vial at room temperature by student t-test at $p < 0.05$.
Expected range of control results was 202 to 274 mg/dL.

temperature, sunlight, and incubator environments remained stable throughout 56 days of testing.[11]

Test strips stored in open vials in the increased light and humidity environments showed the shortest period of stability, failing at 3 and 14 days with control results elevated above the expected range. For both light and humidity, the low control failed at day 3 and the high control at day 14. These results support the manufacturer's warnings about exposure to light and moisture resulting in increased glucose values due to discoloration of the strip's test area. An interesting difference from results obtained in the Gonzales and Kampa study was the stability of test strips stored in the refrigerator environment. While test strips in the Gonzales and Kampa study had the lowest stability period at eight days, refrigerated test strips in the ECU study demonstrated stability for at least 35 days.[11] This difference may have been due to the location of the open vials in the refrigerator in ECU's study. The test strip vials in this study were stored within the main compartment of the refrigerator where temperatures tend to be more constant, while the vials in the Gonzales and Kampa study may have been stored in the shelves of the refrigerator door that would be subject to more temperature fluctuations. The open vials in the incubator were stable until days 14 and 21 for the high and low control, respectively. The closed vials in the incubator and light both lost stability at 28 days.

According to the t-test analyses, the following environments showed a significant difference in mean values from the reference test strips, i.e., closed vial at room temperature, for the low and/or high control(s): open vial at room tem-

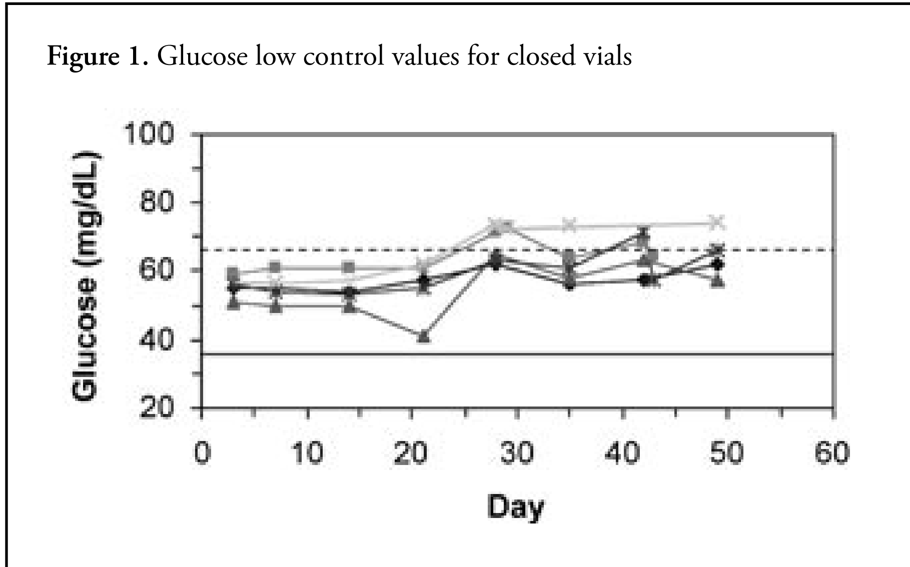


Figure 1. Glucose low control values for closed vials

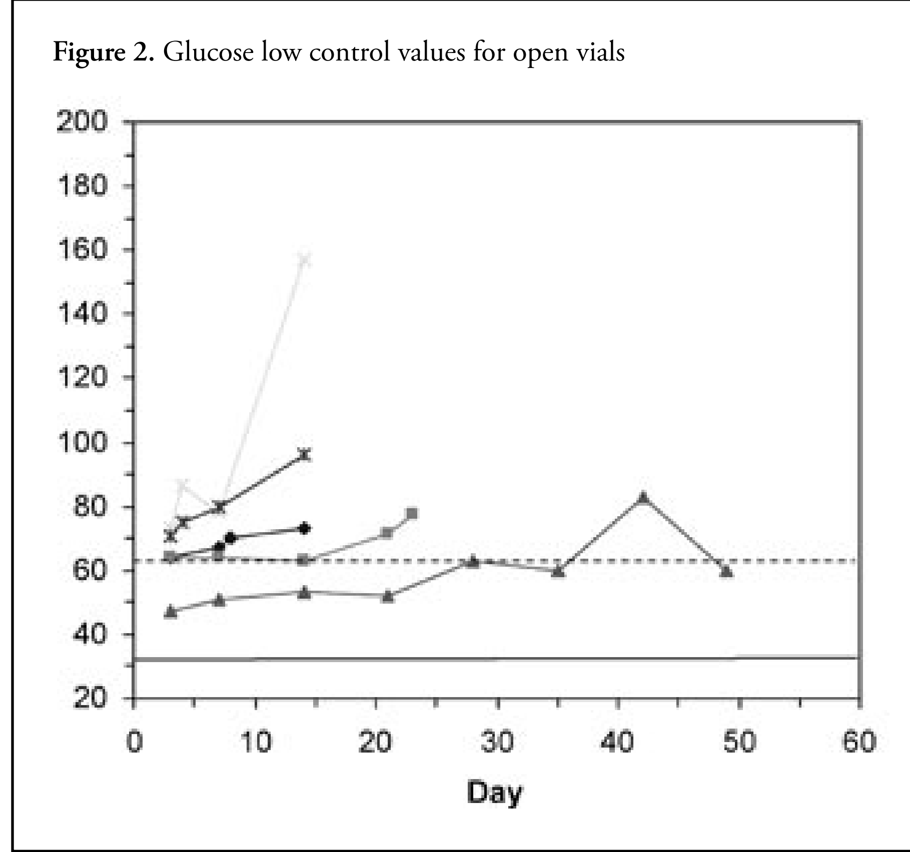
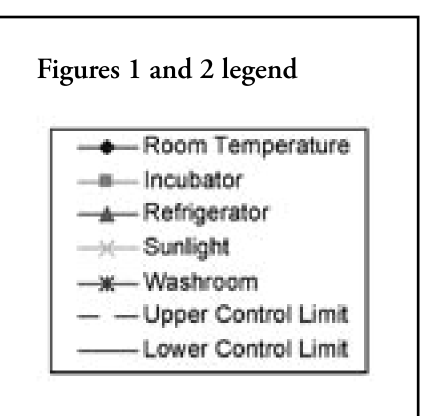


Figure 2. Glucose low control values for open vials



Figures 1 and 2 legend

perature, closed vial in light, and open and closed vials in incubator and refrigerator. For comparison of variances to the values for the closed vial strips at room temperature, the significant differences were predominantly for the refrigerator and light environments.

CONCLUSIONS

The results of this study indicate that glucometer reagent test strips stored in open vials, regardless of storage environment, lose stability more quickly than those in closed vials do. The quickest deterioration in test strip stability was found with open vials exposed to direct light or to humidity. These results support the findings of the most recent similar study as well as the recommendations of the manufacturer to avoid light and moisture which can cause the strips to produce falsely elevated glucose results.[11] The results support the manufacturer's instructions, which state that the test strip vials should be stored tightly capped at room temperature. Other than the recommended storage environment, the study found the overall longest period of stability to be the refrigerator environment for both open and closed test strip vials. This is in contrast to the Gonzales and Kampa study, which found refrigeration of strips to provide the least stability.[11]

The results of this study are limited by several constraints inherent in the study design. Only a limited number of reagent test strips from the same lot number were available from the manufacturer due to discontinuation of the Accu-Check Easy POCT glucose testing system. This prevented the study from evaluating performance of the test strips over a longer period of time, and did not allow for 50 strips in all vials at the beginning of testing. The need to contain the costs of student research projects necessitated doing the study with a glucometer the program

Figure 3. Glucose high control values for closed vials

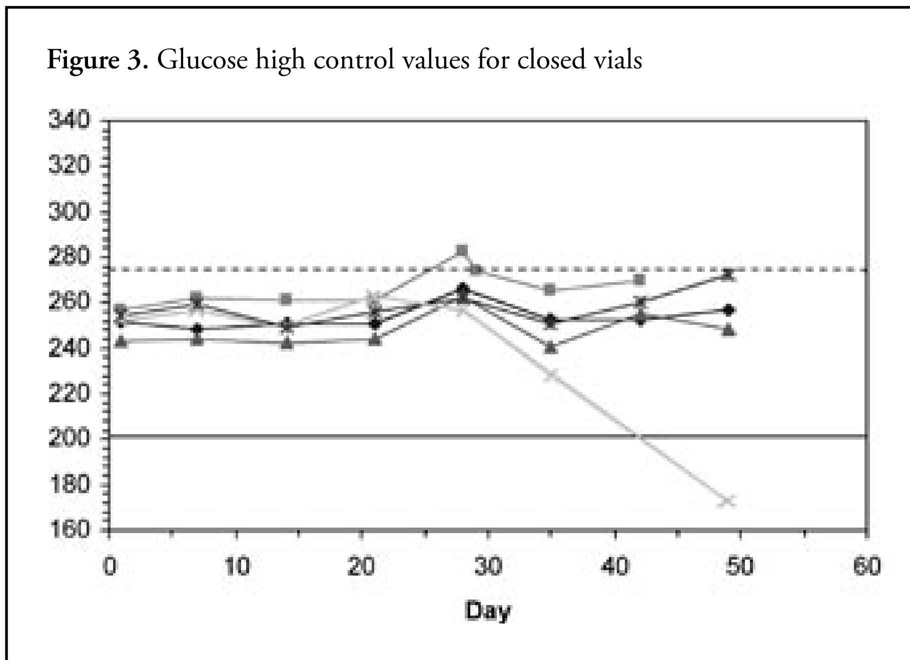
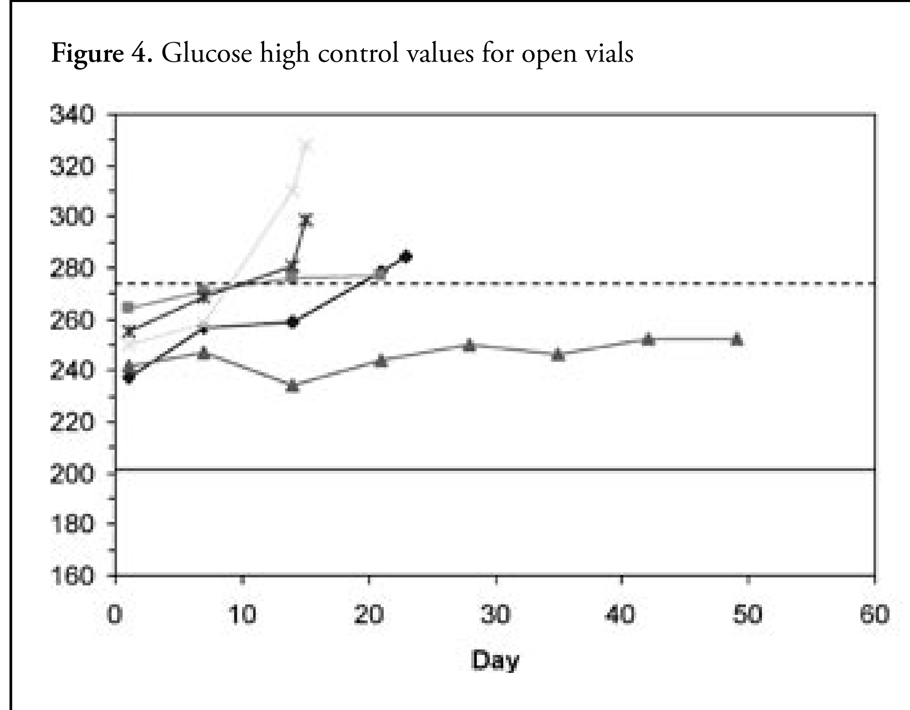
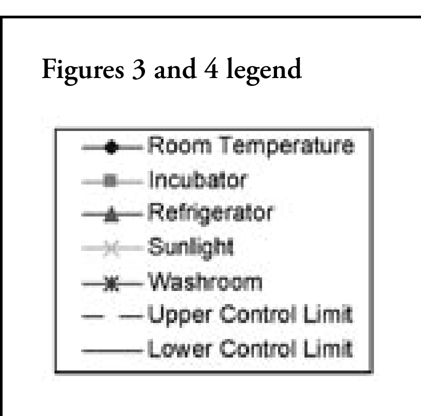


Figure 4. Glucose high control values for open vials



Figures 3 and 4 legend



purchased several years previously, but which has since been discontinued by the manufacturer. Knowing the exact day at which stability was lost was not possible due to the clinical rotation schedule of the students that allowed testing strips only every seven days.

Some newer glucometer models have discontinued the reflectance photometry, i.e., colorimetric, method of glucose reaction detection, and instead use an electrochemical detection system. Some manufacturers have also begun storing test strips individually wrapped in foil for the newer systems.[2,3] Roche Diagnostics, the manufacturer of the Accu-Chek line of glucometers, has converted the glucose test system to an electrochemical measurement and continues to package the test strips in tightly capped vials.[12]

Future studies of the effects of varying storage conditions on the performance of glucometer reagent test strips are warranted on the newer glucometer models using the electrochemical principle of measurement and newer packaging systems. Future research may include longer testing periods that are closer to the expiration periods of the strips, daily testing of strips, multiple storage locations within a refrigerator environment, and financial support from manufacturers to allow a more comprehensive study and a better assessment of test strip viability in adverse storage conditions. As patients do not always precisely follow the manufacturer's instructions, better understanding of test strip stability in alternate storage environments should improve the use of glucose results generated through at-home testing.

REFERENCES

1. Roche Diagnostics. The Roche Accu-Chek Compact System. Available at http://www.roche-diagnostics.com/products_services/accuchek_compact.html. Accessed August 8, 2004.
2. American Diabetes Association. All about diabetes. Available at http://www.diabetes.org/about-diabetes.jsp. Accessed July 28, 2004.
3. Smith T. POCT: from model to method. Adv for Admin Lab 2004;13(4):30-32,34,36-37,40.
4. Sacks DT. Carbohydrates. In Burtis CA, Ashwood ER, editors. Tietz fundamentals of clinical chemistry. 5th ed. Philadelphia PA: WB Saunders; 2001. p 427-61.
5. Devreese K, Leroux-Roels G. Laboratory assessment of five glucose meters designed for self-monitoring of blood glucose concentrations. Eur J Clin Chem Clin Biochem 1993;31:829-37.
6. Gregory M, Ryan F, Barnett JC, Youtz T. Altitude and relative humidity influence results produced by glucose meters using dry reagent strips. Clin Chem 1988;34:1312.
7. Lenhard MJ, Decherney GS, Maser RE, and others. A comparison between alternative and trade name glucose test strips. Diabetes Care 1995;18:686-9.
8. Nichols JH, Howard C, Loman K, and others. Laboratory and bedside evaluation of portable glucose meters. Am J Clin Pathol 1995;103:244-51.
9. Piepmeier EH Jr, Hammett-Stabler C, Price ME, and others. Atmospheric pressure effects on glucose monitoring devices. Diabetes Care 1995;18:423-4.
10. Calderon UJB, Blanco RT, Baltuille FR, and others. The long-term stability of reactive strips for capillary blood glucose preserved at between 2 and 5 degrees C. Atencion Primaria 1993;12:201-4.
11. Gonzales Y, Kampa IS. The effect of various storage environments on reagent strips. Lab Med 1997;28(2):135-7.
12. Roche Diagnostics. Roche Accu-Chek glucose meters. Available at http://www.accu-chek.com/products/meters_meters.jsp. Accessed August 8, 2004.

Clin Lab Sci Seeks Manuscripts

Clinical Laboratory Science encourages authors to submit manuscripts for possible publication in the following areas: inflammatory disease, molecular biology and diagnostics, new advances in technology, interdisciplinary initiatives, and laboratory management, as well as articles focused on a specific discipline. If you have an idea for an article but aren't sure how to begin, *Clinical Laboratory Science* editors can put you in touch with a mentor to help you prepare the manuscript. Editors' contact information can be found on the inside front cover of this issue.

Improving the Accuracy of Specimen Labeling

BOBBI DOCK

Accurate specimen identification is a challenge in all hospitals. A mislabeled specimen can lead to devastating consequences for a patient. In an effort to decrease the risk of potential harm caused by labeling errors, Children's Hospitals and Clinics of Minnesota successfully implemented a Zero Tolerance Laboratory Specimen Labeling process. After months of studying, charting, networking, and communicating with all stakeholders the new process led to a 75% reduction in laboratory specimen labeling errors.

ABBREVIATIONS: FMEA = Failure Mode and Effects Analysis.

INDEX TERMS: specimen labeling.

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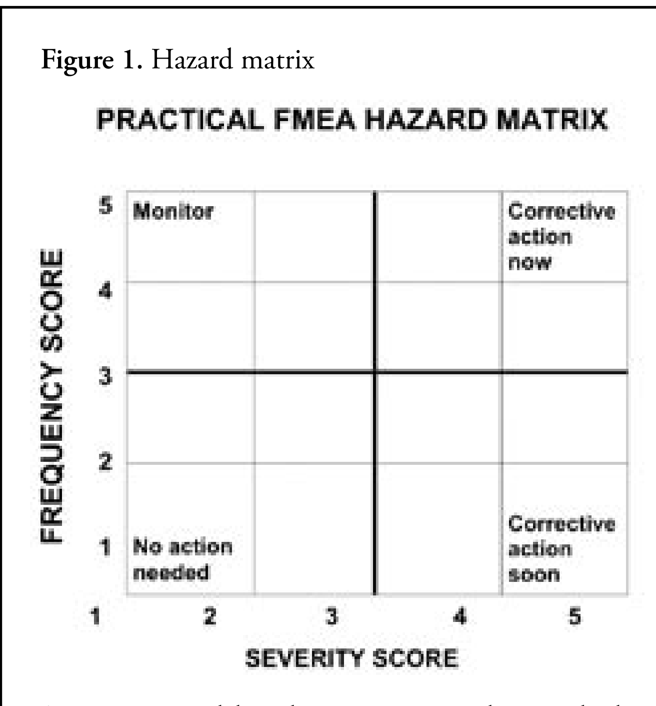
Over 70% of all information used by a clinician to diagnose and treat a patient comes from the laboratory.[1] Ensuring that specimens are correctly identified at the point of collection is essential for accurate diagnostic information. Patient and/or specimen misidentification can be serious, resulting in misdiagnosis and mistreatment.[2,3] A misidentification event creates multiple victims: the patient whose treatment was based on the provided results, the patient whose sample it actually was who may have gone untreated, and the healthcare workers who were directly involved with the patient or the specimen. There are also financial and emotional costs from this type of error. While the financial toll can be calculated, the emotional toll on the patients, their families, and healthcare workers who experience its impact is not easily quantifiable.

METHOD

In April 2003, a multidisciplinary team from Children's Hospitals and Clinics of Minnesota performed a Failure Mode and Effects Analysis (FMEA).[4] The team was composed of representatives from the following departments: the clinical laboratory, pathology, process improvement, nursing, and risk management. FMEA analysis identifies potential flaws *before* an error occurs through an intense scrutiny of a specific process, in this case, laboratory specimen labeling. Initially, the labeling process was observed, charted, and discussed and staff interviews were conducted. Data from these activities were used to construct a

Figure 1. Hazard matrix



A 5 x 5 matrix. Each hazard score represents a risk priority level. This matrix provides guidelines of whether actions should be taken for a particular risk factor.

The peer-reviewed Clinical Practice Section seeks to publish case studies, reports, and articles that are immediately useful, are of a practical nature, or contain information that could lead to improvement in the quality of the clinical laboratory's contribution to patient care, including brief reviews of books, computer programs, audiovisual materials, or other materials of interest to readers. Direct all inquiries to Bernadette Rodak MS CLS(NCA), Clin Lab Sci Clinical Practice Editor, Clinical Laboratory Science Program, Indiana University, Fesler 409, 1120 South Avenue, Indianapolis IN 46202-5113. brodak@iupui.edu.

hazard matrix showing the frequency and severity of an error at each step in the process between ordering a laboratory test and charting a result (Figure 1).

RESULTS

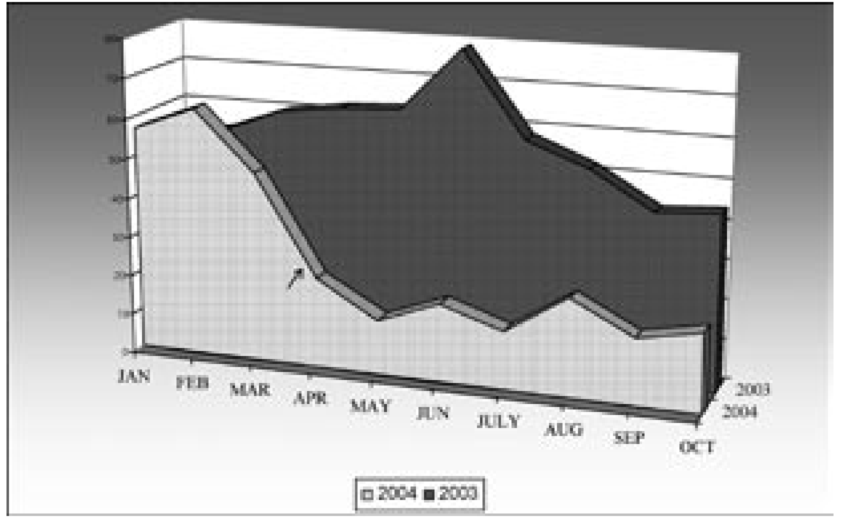
The pre-analytical labeling phase, with approximately two-thirds of the errors, was identified as the key focus area for improvement (Table 2). The FMEA team explored several ways to address specimens that could arrive in the laboratory either mislabeled or unlabeled. Many institutions have adopted an exception list of specimens that, if improperly labeled, can be relabeled and analyzed by the laboratory. The FMEA team considered this process and met with various physician groups to solicit feedback. There was no consensus regarding the proposed exception list. Therefore, that method was discarded and the decision was made that Children's Hospitals and Clinics of Minnesota Laboratory will accept only those patient specimens that meet the Joint Commission for Accreditation of Healthcare Organizations (JCAHO) standards for specimen labeling.[5] JCAHO standards specify two identifiers; Children's uses full patient name and medical record number as acceptable specimen labeling.

Table 1. Number of specimen errors at each stage in the process from ordering a test to charting a result

	Minneapolis	St. Paul	Aggregate
Pre-analytic	453 (70.6%)	488 (63.5%)	941 (66.7%)
Analytic	64 (10.0%)	83 (10.8%)	147 (10.4%)
Post-analytic	89 (13.9%)	106 (13.8%)	195 (13.8%)
Unknown	36 (5.6%)	92 (12.0%)	128 (9.1%)
Total reports	642	769	1,411

Data were obtained during June 2001 through April 2003, prior to implementation of the organizational policy.

Figure 2. Total number of mislabeled/unlabeled specimens arriving at the laboratory each month



The arrow denotes implementation of the Zero Tolerance policy in March 2004.

The FMEA team balanced safe patient care, practical solutions, policies for the staff, and a high level of patient, family, and physician satisfaction in arriving at this conclusion. The organizational policy on laboratory specimen labeling was approved and implemented on March 22, 2004. It is applicable to all laboratory specimens.

The policy does allow for challenging the rejection decision through a process involving the ordering clinician, the healthcare worker who collected and labeled the specimen, and the pathologist. The discussion can result in labeling or relabeling a specimen after it has arrived in the laboratory.

An effective communication strategy was part of the policy implementation process. The FMEA team utilized numerous internal publications to announce the new policy during the month prior to implementation. In addition, warning notices were given by laboratory personnel to staff in areas where mislabeling occurred during this phase.

The results of the new policy have been impressive. Figure 1 shows a 75% decrease in the number of mislabeled/unlabeled specimens received by the laboratory since the policy was implemented. Of the remaining 25%,

the majority were recollected and submitted for testing. Fewer than 40 specimens have been challenged and approved for testing to date, which is 25% of the total mislabeled or unlabeled submitted.

DISCUSSION

Awareness of the potential harm caused by mislabeled laboratory specimens and implementation of a rigorously developed organizational policy led to the success of the Zero Tolerance effort. "Any Is Too Many" is the motto chosen to illustrate our efforts to eliminate the occurrence of mislabeled or unlabeled laboratory specimens. This project is one of many efforts that Children's Hospitals and Clinics of Minnesota is pursuing through its patient safety agenda to ensure a culture of high reliability for patient safety via focused activities that support an attitude of safety.

REFERENCES

1. Garber C. Six Sigma. Its role in the clinical laboratory. Clin Chem News; April 2004:10-4.
2. Nutting PA, Main DS, Fischer PM, and others. Toward optimal laboratory use. Problems in laboratory testing in primary care. JAMA 1996;275(8):635-9.
3. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. Clin Chem 1997;43(8 Pt 1):1348-51.
4. Woodhouse S, Burney B, Coste K. To err is human: improving patient safety through failure mode and effect analysis. Clin Leadersh Manag Rev 2004:18(1):32-6.
5. JCAHO 2004 Comprehensive accreditation manual for hospitals. The official handbook (CAMH)/Update2; May 2004: Chicago. p 182.

CLINICAL LABORATORY SCIENCE
Distinguished Author Award Ballot

The Editors of *Clin Lab Sci* solicit your assistance in selecting the next recipient(s) of the *Clin Lab Sci* Distinguished Author Award. You are invited to participate in the selection process by completing this ballot and sending it to the editorial office no later than February 1, 2006. The award will be presented at the ASCLS annual meeting in July 2006.

ASCLS members, *Clin Lab Sci* readers, and *Clin Lab Sci* editors will choose the recipient(s) of the award. Nominations should be based on originality and quality of writing, relevance to the laboratory science profession, and integration of theory and application.

Please indicate your selection of the best article for 2005 from the four eligible issues of *Clin Lab Sci*, volume 18, issues 1 through 4. The nominated article can be from any section of the journal.

____
Title

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Lead Author

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Volume and Issue

Send this completed ballot to: CLS Editorial Office, IC Ink, 858 Saint Anne's Drive, Iowa City IA 52245

CLINICAL PRACTICE

Piloting Case-based Instruction in a Didactic Clinical Immunology Course

KATHLEEN HOAG, JANET LILLIE, RUTH HOPPE

OBJECTIVES: To assess 1) the effect of case-based instructional modules on student critical thinking, class attendance, and satisfaction and 2) student opinion of case formats.

DESIGN/SETTING/PARTICIPANTS: University-based upper division course in clinical immunology and serology. The course was taught by the same instructor for two consecutive semesters with the intervention introduced in the second semester. Sixty-seven students experienced the intervention and 56 students were in the baseline cohort.

INTERVENTION: Nine cases were interspersed between lectures during the semester. Each case took one 50-minute class in which students worked in groups of five or six.

MAIN OUTCOME MEASURES: Student performance on five critical thinking multiple-choice examination questions and percent student attendance on case days versus lecture days were analyzed using the Mann-Whitney test. Student ratings on course evaluations were analyzed using t-test comparing semesters with and without intervention. Student opinion of cases was obtained through surveys and a focus group.

RESULTS: Student performance on critical thinking exam questions was similar in the two groups. Student attendance was significantly higher on case days (95.6%) versus lecture days (80.3%; $p<0.0001$). Only composite ratings for instructor involvement, student-instructor interaction, and course organization were significantly improved in the semester with cases compared to the semester with lecture only ($p<0.0001$ for all indices).

CONCLUSIONS: Although case studies did not significantly improve student performance on critical thinking questions, they still proved to be a valuable instructional method. Student attendance, student-instructor interaction, and instructor involvement in the course were all positively affected by incorporation of case studies. Discussion of cases also helped to uncover student misconceptions of course material.

ABBREVIATIONS: CL = cooperative learning; CLS = clinical laboratory science; Ig = immunoglobulin; PBL = problem-based learning; SIRS = Student Instructional Rating System.

INDEX TERMS: active learning; case-based instruction; cooperative learning; immunology.

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Lecturing is the predominant form of higher education instruction worldwide, yet it has some flaws that concern educators.[1] Lecturing is a teacher-focused method of instruction characterized by passive information delivery that typically fails to engage students. Students often take notes without processing the information, tending only to record random facts that they believe they need to memorize for an examination. Further, instructors have all experienced students who sleep, read newspapers, work crossword puzzles, or are otherwise completely disengaged with a lecture. Additionally, the average adult learner's attention lapses dramatically 10 to 18 minutes into a lecture, with lapses in attention occurring more frequently throughout the average 50 minute lecture.[2] Most important, compared to active-learning techniques, traditional lecturing fairs poorly in student retention of information and development of student critical thinking and life-long learning skills.[3] Despite these known deficiencies, lecture-based instruction is often maintained for a number of possible reasons: 1) faculty familiarity with what they experienced as undergraduates; 2) peer-pressure from colleagues who have always lectured; 3) ignorance of alternative instruction methods; 4) lack of institutional support for training in alternative instruction methods; 5) lack of published research demonstrating advantages of alternative instruction method in their particular discipline; or 6) economical savings of low faculty to student ratio in high enrollment lecture-based courses.

Problem-based learning (PBL) and cooperative learning (CL) in the form of patient case-based instruction are alternatives to lecturing that have gained considerable popularity in health science education, especially in medical school curricula. PBL and CL are similar methods of instruction that differ mostly in complexity and level of responsibility placed upon the learner. Both PBL and CL use learners working together in groups to accomplish tasks or activities. CL has been defined as "students working together to accomplish shared learning goals and maximize their own and their groupmates' achievement".[4] CL techniques stress positive interdependence and try to eliminate student competition, especially within groups.[4] The activities used for CL can be quite varied and have been summarized elsewhere.[4,5] PBL is a highly advanced form of CL. Whereas CL can be used exclusively as an instructional method or alternatively integrated into lectures as enhancement activities, some strong advocates of PBL believe it should be used as the exclusive course delivery method.[6]

In health sciences education, PBL generally uses real-life patient cases as the focus of learning. In its purest mode, the responsibility for learning is placed on the students and the instructor is only responsible for presentation of a clinically-relevant problem and facilitation of student learning.[7,8] Key features of PBL are patient case studies that are generally loosely structured and require the learners to use a preexisting knowledge base. The students analyze, research, and discuss initial case information, decide what the appropriate learning objectives will be, and then research and revise learning objectives until the case can be completed.[7-9] For a more thorough discussion of passive and active learning and justification for use of CL and PBL in clinical laboratory science (CLS), the reader is referred to McEnerney (1999).[10]

The theoretical and practical application of CL and PBL to CLS education has been appreciated for more than a decade.[11-13] The goals of CLS instructors in choosing to use CL or PBL are varied and include: 1) increasing student achievement; 2) increasing student critical thinking and/or problem solving skills; 3) promoting teamwork skills; 4) fostering life-long learning skills; and 5) increasing knowledge retention.[5,14-17]

The overall design of this pilot study was to incorporate case-based activities into a lecture course and assess the effect on several indicators of student performance and student satisfaction. The intervention reported here uses the patient case-based learning format of PBL but really should be considered CL as it falls short of the strict definition of PBL for two reasons.[7] First, the cases were not used exclusively as the only instructional method in the course, but were instead used to augment lectures with the intention of connecting concepts that the students were taught previously as discrete lecture elements. This was deemed necessary for this course since it is the only immunology course in the undergraduate curriculum and the students did not have a significant knowledge base to draw upon. Second, the students did not choose the learning objectives based upon the patient case presentation but instead were given specific questions by the instructor to answer within a single class period. Therefore, the most appropriate categorization is what Barrows refers to as the "case method" and what we will refer to as CL cases.[9]

The primary goal of this study was to assess whether CL cases would improve student performance on critical thinking level examination questions that required clinical application of basic immunology concepts. A secondary objective of the study was to collect student opinion of case format and implementation for subsequent optimization of case design within the course. Additionally, the instructor hoped to improve student attendance with the use of CL cases. Student performance was assessed on five analysis/application level multiple choice exam questions. Student attendance on CL case days was compared to attendance on lecture days for the intervention semester only. Data from student course evaluations of course/instructor satisfaction were compared for semesters with and without case-based CL intervention. Finally, subjective student evaluation of CL case design and implementation was collected by the use of a student survey and a retrospective focus group.

METHODS

Overall study/course design

The study of small group discussion of patient cases took place in a semester-long 3-credit junior/senior level undergraduate course in clinical immunology and serology with 67 students. The study was partially retrospective to a previous semester (the baseline semester) that was taught by lecture-only format using PowerPoint® presentations to a group of 56 students. In the subsequent semester, nine patient case studies were incorporated into this course as the intervention. The study was approved by the Michigan State University Committee on Research Involving Human Subjects. Students who took the course during the intervention semester were informed of the study and asked to sign a consent form. All except one student agreed to participate in the study. The course was taught during a standard 15-week semester, and met Monday, Wednesday, and Friday of

each week for 50-minute class periods. Four hourly exams were administered during the semester. Thirty class periods were used for standard lecture presentation of material. The initial one-quarter of the course material (prior to the first hourly examination) was presented using lecture only without cases. After the first examination, cases were scheduled for every Friday, with each case being related to the lecture material presented on Monday and Wednesday of the same week (when possible). Overall, nine case sessions were held. Detailed lists of learning objectives were provided for each lecture. Cases did not have specific learning objectives and material presented only via cases (and not in lecture) was not assessed on examinations.

Student group format

Twelve groups of five or six students each were assigned by the instructor and were fixed throughout the semester. Students were assigned to groups based upon incoming grade point average (distributed among groups), race, and gender. An effort was made not to leave any given student a 'single' in a group, e.g., only Caucasian female. Groups handed in only one answer sheet and each student in the group was required to sign the sheet verifying that they agreed with the answers and had actively participated in generation of the answers. One student had the role of 'note taker' each week and this role was required to rotate among group members from case to case. To encourage and account for balanced group member participation, each student had to assess fellow group members using a standard assessment form following every third case (peer assessment).

Case study content and design

The patient case studies were presented using a standard informational format and one of three possible delivery formats (see below). Cases were adapted from previously published case studies.[18,19] Each case study had a patient identified by name, age, and gender. The patient history was given in a one to two paragraph summary that included presentation symptoms, physical examination details, and laboratory tests ordered. Family history was also provided if appropriate. Results of the laboratory tests were displayed on an overhead projector throughout the class period. Since laboratory testing was not covered in lecture, the instructor provided reference books that the students could use during class to investigate the laboratory tests and their reference ranges and significance. Each group then received one of four possible question sets provided in hard-copy. Group members had their desks arranged in circles to facilitate group conversation (approximately 30 to 40 minutes). Five to ten minutes were retained at the end of the class period to allow for instructor debriefing of the most important questions the groups had addressed. Each group answer was worth a possible five points and case study grades accounted for 13% of the course grade. Each group member received the same grade as other members of that group. Students with excused absences were allowed to answer the case study questions individually outside of class for credit. Graded answers with comment were photocopied and returned to the students as soon as possible, usually by the next class session.

Example case

The following example case is provided so that the reader may understand the working definition of critical thinking application questions as used in the CL cases. This case is an example of agammaglobulinemia (the students were not given the diagnosis).

A two year old boy (Bill) presented to a pediatrician with pneumonia, recurrent bacterial infections, and no visible tonsils or palpable cervical lymph nodes upon physical examination. Laboratory results included a complete blood cell count with white blood cell differential (all normal) and total serum antibody results for immunoglobulin (Ig) G, IgA, and IgM. Serum antibody results were: IgG 200 mg/dL (reference range 600-1500 mg/dL); IgA <1 mg/dL (50-125 mg/dL); and IgM 10 mg/dL (75-150 mg/dL). The following are representative questions the students were asked to answer in groups:

1. List the abnormal laboratory test results.
2. Explain the differences in the degree of deficiency for the three serum antibody isotypes tested.
3. Based upon your answer for question 2, can you explain why Bill did not get ill until he was 10 months old?
4. Based upon the laboratory results, is it possible that Bill is missing a key white blood cell type? Identify this cell.
5. List as many possible protein mutations that could lead to the suspected deficiency.
6. Suggest the basis for the lack of detectable tonsils and cervical lymph nodes.
7. Suggest further laboratory testing that may be useful in determining the cause of Bill's recurrent infections.

Case study delivery format

The case delivery format was varied during the semester. The formats were: 1) students were read the case history by the instructor at the beginning of class, the laboratory results were presented on an overhead projector, and three to four topic-focused questions per group were handed out

to be completed in 30 minutes; 2) students were read the case history by the instructor at the beginning of class, the laboratory results were presented on overhead projector, and one in-depth question was handed out to each group to be completed in 40 minutes; or 3) students received a photocopy of the case history and laboratory results one week in advance of group discussion of the case, and three or four topic-focused questions per group were distributed at the beginning of class to be completed in 40 minutes. The slightly different formats were rotated in sequence across the nine Friday sessions utilized for CL.

Data collection and statistical analysis

Student critical thinking skills with and without case study intervention were assessed with five multiple choice examination questions that were repeated verbatim in both semesters (examinations are not returned to students). The questions were categorized as critical-thinking-type questions since they required analysis and/or application of course concepts and could not be answered by memorization. One of the critical thinking questions used was:

> A patient presents to his/her physician with recurrent infections caused by extracellular bacteria. The patient does not appear to be susceptible to other infections. What immune defect would likely be the cause?
>
> a. Defect in antigen presenting dendritic cells.
> b. Defect in class I expression on all body cells.
> c. Defect in class II expression on thymic cortical epithelial cells.
> d. Antibody deficiency due to B cell deficiency.

Individual student performance (number correct out of five possible) for all students in the baseline group and the intervention group was recorded. Statistical analysis was performed with a Mann-Whitney nonparametric test for differences between the medians. A t-test could not be performed because the data were not normally distributed. Statistical significance was set at $p < 0.05$ for this and all other statistical analyses.

Student opinion of the impact of case studies on increasing student level of comfort with course material and student understanding of course material was surveyed four times during the intervention semester (with each examination). A Likert scale ranging from 1 (superior) to 5 (inferior) was used for recording student opinion. The scores (mean and SD) for the four surveys were used to perform linear regression analysis.

Student Instructional Rating System (SIRS) surveys used for campus-wide course/instructor evaluation were used to assess the effect of case studies on overall student satisfaction with the course. The SIRS consists of 21 questions assessed by a Likert scale ranging from 1 (superior) to 5 (inferior) and provides composite profile factor scores for multiple questions related to a theme (instructor involvement, student interest, student-instructor interaction, course demands, and course organization). In this study, composite profile factor scores were compared for semesters without and with intervention using an unpaired t-test. Additional survey questions pertaining to case studies were added to this survey for the intervention semester only, with forced response choices listed (3 to 5) for each question (non-Likert scale).

Aggregate student attendance was taken by head-count 10 to 15 minutes into the class period to allow for latecomers. Percent attendance on days of scheduled lecture versus days of scheduled case study work was compared by the Mann-Whitney test. A t-test could not be used since the variance in the groups was not equal.

A representative sample of students (13 of 67 total students) from the semester of the CL case intervention was recruited to participate in a focus group. The focus group was conducted by a professional with considerable previous experience with moderating focus groups, but who was previously unknown to the students. The instructor was not present at the focus group, but students were aware that the session was audiotaped, and would be transcribed without speaker identification, for documentation purposes.

RESULTS

Class attendance

Daily aggregate class attendance was recorded 10 to 15 minutes into the class period for each day during the semester with CL case intervention. Class attendance on CL case days was significantly higher than on lecture days (Figure 1).

Critical thinking skills

The primary goal of adding the CL cases to the course was to improve student critical thinking skills. Student performance on critical thinking examination questions for the intervention semester (lecture + CL cases) was not significantly different from the baseline semester (lecture only; Table 1).

Student opinion of case impact

Student surveys were administered several times during

the semester to obtain student opinion of the effect of the case studies on two aspects of course material acquisition. Student-reported opinion of their comfort level with course material improved significantly during the course (2.66 to 2.24; r^2 = 0.907; p = 0.047). Student opinion of understanding of course material attributed to the case study also improved, but was not statistically significant (2.55 to 2.28; r^2 = 0.812; p = 0.098) (Figure 2).

Mean scores on end-of-course student evaluations of satisfaction with course/instructor were compared for the semesters with and without CL case intervention. Composite scores for three of five profile factors were significantly higher for the semester with CL case studies compared to the semester without case studies (Table 2). The scores for instructor involvement, student-instructor interaction, and course organization improved significantly for the intervention semester compared to the baseline semester. Scores for student interest and course demands did not change significantly.

Student case format preference

Student opinion of case delivery preference and case utilization was obtained through forced-response survey questions and a focus group. Cases were delivered using three formats during the semester. Students overwhelmingly preferred the format in which patient case background was provided one week in advance of in-class scheduled group work sessions (Table 3). Open-ended questions in the focus group on strengths and weaknesses of the cases and case design revealed that: 1) students valued the case activity because it allowed them to observe peers' thought processes; 2) answering case questions forced students to clarify the material better than they would if they only studied on their own; and 3) peer evaluation was important to ensure the group work was equitable. Students in the focus group also commented on

Figure 1. Mean percent class attendance was significantly higher on scheduled CL case days versus scheduled lecture days during the intervention semester

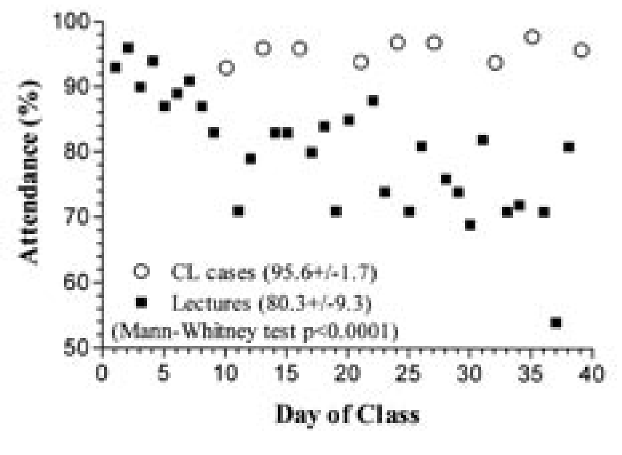
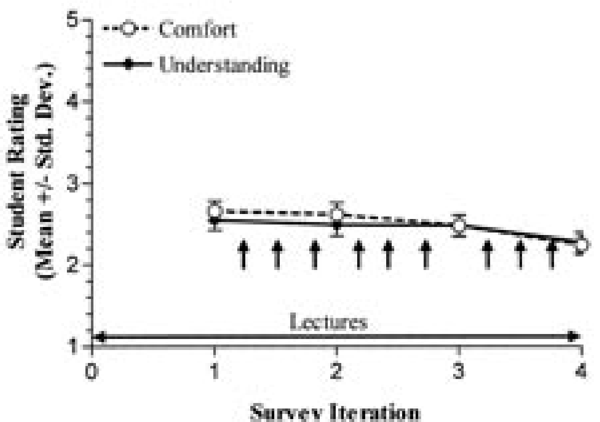


Table 1. Student performance on multiple choice critical thinking exam questions

	B*	I†
Students examined (n)	53	66
Mean‡	3.132	3.106
SD	0.921	1.025
p value§		0.798

* Baseline semester(lecture only)
†Intervention semester (lecture + CL cases)
‡ Number correct out of five total questions
§ Mann-Whitney nonparametric test for differences between medians

Figure 2. Student confort level with and understanding of material



Self-reported student comfort level with course material improved significantly during the intervention semester, but student-reported understanding of course material was not significantly affected during the intervention semester. Students were asked the following questions to address comfort level and understanding: 1) To what degree do the small group exercises increase your level of comfort with the course material? 2) To what degree do the small group exercises increase your understanding of the course material? A Likert scale of 1 (superior) to 5 (inferior) was used for the survey which was administered in 4 iterations during the semester. CL case study occurrences are indicated by ↑.

the cases being scheduled on Friday, and that this allowed a welcome change of pace. However, they also commented that if the cases were not mandatory (points assigned toward grade), they would not have attended on Friday. Instructor observations of case studies are summarized in Table 4.

DISCUSSION

The primary impetus for addition of CL cases to the course was to promote student acquisition of critical thinking skills through the use of active learning techniques. Analysis of student performance on critical thinking level examination questions suggests that this goal was not achieved since there was no significant difference in student performance on these questions for the baseline and intervention semesters. However, the analysis is limited in power since only five questions were used to compare critical thinking skills. This limitation was due to the pool of critical thinking level examination questions available for reuse from the baseline semester to the intervention semester. Since the instructor did not intentionally teach critical thinking skills in the baseline semester, there were few such questions used on the examinations for that semester. Additionally, the assessment questions used (multiple choice) did not match the format of the CL cases, in which the students were asked to draft short written answers to open-ended questions. This mismatch in instruction and assessment formats as well as the limited power of the measure may have limited the ability to detect possible true differences in critical thinking skills that may have been fostered by the CL cases.

A drawback of the current implementation of CL cases that may have interfered with the development of student critical thinking skills was the limited time for students to review and critique their group answers to the case questions. In particular, they had very little time to compare their group answers to those of their peer groups which had the same question sets. In addition, the students were not given an opportunity to critique and revise any of their answers following open class discussion of the questions. The cases were completed in one class session and the following class session was either a lecture or examination with no reference back to the CL case. Well-designed and executed PBL has adequate time for reiteration and reflection that is thought to be essential to ensuring recall and application of the information

Table 2. Composite scores for indicators of student satisfaction

Composite profile factor	Baseline semester (lecture only) mean/SD	Intervention semester (lecture + CL cases) mean/SD	*p* value
Instructor involvement	1.89/0.20	1.63/0.16	<0.0001
Student interest	1.83/0.12	1.88/0.21	0.20
Student-instructor interaction	2.15/0.19	1.90/0.13	<0.0001
Course demands	2.31/0.08	2.28/0.07	0.11
Course organization	2.05/0.19	1.77/0.19	<0.0001

Composite scores for three of five indicators of student satisfaction with course/instructor improve significantly in intervention semester with CL case studies (1 = superior, 5 = inferior; mean/SD reported).

Table 3. Students preferences for case delivery*

Case delivery format	Student preference (%)
Case read aloud, three to four topic-focused questions, 30 minutes to answer	2
Case read aloud, one in-depth question, 40 minutes to answer	27
Cases handed out one week prior, three to four topic-focused questions, 40 minutes to answer	59

*Percent is <100 because 12% of students did not answer the survey question.

to new but similar problems.[7] Therefore, the lack of time for reiteration and reflection may have prevented gains in student critical thinking skills.

The lack of significant effect of CL cases on student critical thinking skills reported here is not completely unexpected. Other reports on studies of CL or PBL effect on student critical thinking skills for courses within CLS curricula have varied in results. Two of these studies had design limitations. One of these studies used groups only for completion of course examinations instead of for student "knowledge construction".[16] This is not a generally accepted method of CL and may have hampered any intended benefit of group work. The second study in which student examination performance was analyzed was limited by two factors.[17] First, the PBL activity was only six weeks in duration and was not continued through the following year of instruction prior to examination. Second, the data presented were from student performance on certification examinations in which the majority of questions are recall level and do not test critical thinking skills. A third study by Bose was well-designed and rigorously used CL techniques but failed to show any significant difference in mean examination scores of students instructed with CL versus lecture.[5] Additionally, this same study failed to show any differences in students' perception of teamwork knowledge, skills, and ability.

Positive effects of PBL in CLS education are limited to two reports of subjective faculty evaluation of student critical thinking skills and learning motivation.[14,15] Although faculty who use CL and PBL generally believe that these instructional methods enhance student critical thinking skills, the studies reported here and elsewhere do not seem to support that view. However, we support an alternative conclusion, which is that these studies failed to show significant differences in critical thinking attributable to CL or PBL because the measures used to assess critical thinking skills were inadequate. Since discipline specific critical thinking skills require discipline specific knowledge, students may answer a question designed to measure critical thinking skills incorrectly if they lack the relevant knowledge.[3] It may be that significant gains in critical thinking skills have been made in CL or PBL versus lecture, but we have been unable to adequately assess those skills.

Table 4. Instructor observations on the use of CL cases

- Cases in groups made it exciting to be in the classroom
- Case discussion assisted identification of student misconceptions
- Overall class attendance increased even on lecture days
- Case studies got students talking and initiated more questions in lectures
- Proper design and implementation of CL cases is time-consuming, but worth it

The current study shows that class attendance was significantly higher on CL case days compared to lecture days. It may also be observed that class attendance decreased as the semester progressed and that attendance tended to be lowest on Fridays. General observation by the instructor also suggests that attendance on lecture days was improved compared to previous semesters. However, this point cannot be objectively tested since attendance was not recorded for the baseline semester, and the class met at a different time of day for the baseline semester. Although a statistical difference in attendance was demonstrated for case days compared to lecture days for the intervention semester, one must keep in mind a serious confounding factor: the students received 13% of their course grade from case studies, and attendance and group participation was mandatory to earn these points. Indeed, students commented in the focus group that many would have skipped class on Friday if case study participation were not mandatory. In retrospect, it seems that scheduling of the cases on Fridays was fortuitous. Since the instructor was hoping to increase student attendance with the use of CL cases, future use of CL cases will continue to encourage CL case participation through point incentives and to schedule cases on Fridays.

An additional and not wholly unexpected benefit of CL cases was significantly improved composite course/instructor ratings for instructor involvement and student-instructor interaction. Traditional lectures do not encourage students to ask questions or comment on the material in the classroom. The small group format of the CL cases may allow students to crystallize concepts and questions in their group with their peers prior to consulting the instructor, if that was deemed necessary. Once a dialog was initiated in the small groups, it appeared that students were freer to ask questions during the lecture and outside of their groups, thereby improving overall student-instructor interaction and the ability of the instructor to become more involved with the student's learning.

The opportunity to discuss course material in small groups of students also appeared to improve student perception of their comfort and understanding of course material. Student opinion of their comfort and understanding due to CL cases improved

during the intervention semester when several iterations of the same survey were administered with three CL cases occurring between each survey iteration. This improvement was only statistically significant for student comfort with course material and not student understanding of course material. However, it should be noted that caution must be used in interpretation of significance for the linear regression analysis presented, as there is a possibility of a type I error since responses of individual students could not be tracked over time (surveys were anonymous). Additionally, the students reported relatively positive effects of CL cases for both comfort and understanding before any CL cases had actually taken place (they were verbally instructed to anticipate the effect of the CL cases on these aspects of the course). This may indicate that the students viewed the CL cases relatively positively even before they had experienced them, and this positive opinion was upheld once they had experienced them. An equally possible explanation is that the lecturer was very skilled and that the students became more comfortable with the course material during the course of the semester due to superb lecturing that was unrelated to the CL case experience. However, student comments during the focus group session support the former explanation more than the latter, or a combination of both.

One of the limitations of the reported CL case design discussed above was the lack of time for student reiteration and reflection. This was viewed by the instructor as a significant flaw in the CL cases. As a result, when CL case activities are used again for the same course, the instructor plans to devote two consecutive class sessions to each CL case. The first class session will have the students meet in groups to answer the case questions (as currently described). The second additional class session will entail class-wide discussion of student group answers to all of the questions followed by 10 to 15 minutes for groups to revise their written answers to their questions for additional points.

Although this study found that utilizing CL cases had no effect on student critical thinking skills, the instructor is still enthusiastic about using this instructional method again. Due to the limitations in the way in which critical thinking skills were assessed in this study, it may be that student critical thinking skills did indeed improve and that we were unable to detect it. Additionally, student comments in the focus group were very positive regarding the CL cases. The majority of students enjoyed working with their peers and valued the CL cases for a variety of reasons. The instructor also enjoyed the CL case activities and observed several advantages of the instructional method (listed in Table 4), some of which were not anticipated prior to use of the CL cases. As a result, the CL cases will continue to be used in upcoming semesters with some minor modifications to the design as discussed above.

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REFERENCES

1. McKeachie WJ. Teaching tips: strategies, research, and theory for college and university teachers, 10th ed. New York: Houghton Mifflin Company; 1999.
2. Johnstone AH, Percival F. Attention breaks in lectures. Educ Chem 1976;13:49-50.
3. McKeachie WJ, Pintrich PR, Lin Y-G, and others. Teaching and learning in the college classroom: a review of the research literature, 2nd ed. Ann Arbor: NCRIPTAL, University of Michigan; 1990.
4. Johnson DW, Johnson RT, Smith KA. Active learning: cooperation in the college classroom. Edina, MN: Interaction Book Company; 1998.
5. Bose MJ, Jarreau PC, Lawrence LW, Snyder P. Using cooperative learning in clinical laboratory science education. Clin Lab Sci 2003;17(1):12-8.
6. Charlin B, Mann K, Hansen P. The many faces of problem-based learning: a framework for understanding and comparison. Med Teach 1998;20(4):323-30.
7. Barrows HS. The essentials of problem-based learning. J Dent Educ 1998;62(9):630-3.
8. Miller SK. A comparison of student outcomes following problem-based learning instruction versus traditional lecture learning in a graduate pharmacology course. J Am Acad Nurse Pract 2003;15(12):550-6.
9. Barrows HS. A taxonomy of problem-based learning methods. Med Educ 1986;20(6):481-6.
10. McEnerney K. Active learning and situational teaching: How to ACE a course. Clin Lab Sci 1999;12(1):35-41.
11. McEnerney K. Cooperative learning as a strategy in clinical laboratory science education. Clin Lab Sci 1989;2(2):88-9.
12. McEnerney K. Cooperative learning in clinical laboratory science education. Clin Lab Sci 1994;7(3):166-71.
13. Doig K. Problem-based learning: Developing practitioners for today and tomorrow. Clin Lab Sci 1994;7(3):172-7.
14. Beadling W, Vossler J. Problem-based learning in the clinical laboratory science curriculum. Lab Med 2001;32(8):442-51.
15. Hesse DE, Hentzen AE. Reverse problem based learning (R-PBL). Lab Med 2002;33(3):218-21.
16. Milson LM, Laatsch LJ. Does cooperative learning enhance student achievement? Lab Med 1996;27(9):618-21.
17. Teshima DY. Outcome measurement of problem-based learning. Clin Lab Sci 2001;14(2):68-9.
18. Rosen FS, Geha R. Case studies in immunology: a clinical companion, 3rd ed. New York: Garland Publishing; 2001.
19. Stevens CD. Clinical immunology and serology: a laboratory perspective, 2nd ed. Philadelphia: FA Davis Company; 2003.

Screening for Diabetes: Sensitivity and Positive Predictive Value of Risk Factor Total

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OBJECTIVE: Screening for diabetes is recommended for individuals ≥45 years of age, or earlier if they manifest ≥ one specific risk factors. This study examined the sensitivity and positive predictive value (PPV) of risk factor total for identifying individuals with diabetes and prediabetes.

DESIGN: Subjects were interviewed to assess the presence of risk factors. Fasting plasma glucose levels were obtained.

SETTING: The study occurred at a health fair in Greensburg, PA.

PATIENTS: Six hundred sixty-one Caucasians between the ages of 19 and 100.

RESULTS: Using the criterion of screening individuals with ≥ one risk factors detected 100% of both diabetics and prediabetics. This dropped to 91.2% when screening individuals with ≥ two factors. The PPV of the risk factor total was poor (80% of individuals with a total of four factors were not diabetic). The ability of the risk factor total to predict individuals with impaired glucose metabolism (prediabetics + diabetics) was considerably better, and increased almost linearly with the risk factor total. Of the subjects with normal glucose values, the mean glucose increased as the risk factor total increased.

CONCLUSION: While the sensitivity of using ≥ one risk factor as an algorithm to screen is 100% for identifying diabetics, the PPV of risk factor analysis for identifying diabetics is poor. The same algorithm works well to identify at-risk individuals, presumably allowing early intervention and education.

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ABBREVIATIONS: FPG = fasting plasma glucose; PPV = positive predictive value.

INDEX TERMS: diabetes mellitus; euglycemia.

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Hyperglycemia is a risky business. Elevated glucose levels in diabetes mellitus are associated with the risk of retinopathy, kidney failure, and neurologic damage.[1] Coronary vascular disease is strongly associated with diabetes; in fact, it is the leading cause of diabetes related deaths.[2] An individual with diabetes has the equivalent risk of suffering a major coronary event as a person who already has coronary heart disease.[3] An increasing number of Americans are exposed to this risk: 6.3% of the U.S. population can be classified as diabetics.[4] A diagnosis of diabetes can be made three ways. The first is by an individual having the classic symptoms of diabetes, e.g., polydipsia, polyuria, and unexplained weight loss, and a casual glucose value ≥11.1 mmol/L (200 mg/dL). The second is by an individual having a fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL), confirmed by repeat testing on a different day. The third method is a two-hour post glucose load value ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test, repeated on a different day.[5] The cutoff values are based on the increased incidence of retinopathy that is associated with glucose values at or above these levels. There is no threshold glycemic value that predicts macrovascular risk.

Individuals who have type 2 diabetes may already bear some of its 'scars' at the time of diagnosis. Newly detected type 2 diabetics have a higher degree of early atherosclerosis than non-diabetics.[6] Nephropathy may be found at this time as

well; therefore, newly diagnosed diabetics should be screened for microalbumin.[1] Frank diabetes follows a 'prediabetic' period. Recently, the FPG range that defines this stage has been lowered to include 5.6 mmol/L to 6.9 mmol/L (100 mg/dL to 125 mg/dL); 21.1% of Americans age 40 to 74 fall into this new range.[4,7] Individuals with prediabetes have a relative risk of heart disease of 1.19 to 1.33 compared to euglycemic individuals.[8,9] The benefit of classifying an individual as prediabetic is to intervene and attempt to prevent subsequent diabetes. Several studies have shown that life style changes and/or pharmacologic therapy targeted at prediabetics can substantially reduce the incidence of progression to diabetes.[10]

The American Diabetes Association does not recommend screening the general population for diabetes, but instead uses a risk-based approach. Since age is a risk factor in becoming diabetic, screening should begin at age 45, and be performed every three years thereafter.[11] Other factors that are associated with increased risk of developing diabetes are being overweight (body mass index ≥25 kg/m^2), family history of diabetes, race other than Caucasian, hypertension (even if treated), dyslipidemia, history of gestational diabetes or delivering a baby > nine pounds, and habitual physical inactivity. Individuals that have one or more of these risks should be screened for diabetes at a younger age.[11] The correlation between risk factors and diabetes is not expected to be 100%, but the greater the number of risk factors, the greater the chance is that an individual has or will develop diabetes.[11] Risk factor assessment is a simple method for prediction, does not weight any factor higher than another, and does not take into account possible interactions between the factors. Its advantage is that it is easy to perform, all factors have an equivalent risk, and they are summed. If an individual has one or more risk factors, the physician may decide to screen the individual for diabetes before their 45th birthday.

This study enrolled a large group of Caucasian men and women who attended a community health screen, and correlated the risk factor total to the prevalence of diabetes and prediabetes in that population to determine the sensitivity and PPV of risk factor total as a screening tool for diabetes. This study also examined the level of FPG in euglycemic individuals with respect to risk factor total.

MATERIALS AND METHODS

Subjects were solicited at a health fair in Greensburg, Pennsylvania in April 2003. Six hundred sixty-two people (264 males and 398 females) enrolled in the study. Each completed a health survey and provided informed consent. Subjects ranged in age from 19 years to 100 years, with a mean of 60.6 (SD 14.0) and a median of 61. All of the subjects were non-Hispanic Caucasians except for one African-American man. Because race other than Caucasian is a known risk factor for diabetes, and only one participant was not Caucasian, he was excluded from this study. Survey materials were approved by the Institutional Review Board at the University of West Florida. There was no financial incentive for participation. Participants were fasting 10 to 12 hours prior to phlebotomy.

Samples were collected in lithium heparin tubes with separator gels and centrifuged within one hour of collection. Testing was performed on a Roche Hitachi Modular® Analytics Instrument (Roche Diagnostics Corporation) at Westmoreland Regional Hospital in Greensburg PA by a qualified technical staff. The population was sorted into three groups using FPG value and history of diabetes: nondiabetics with FPG <5.6 mmol/L (99 mg/dL) were classified as euglycemics, nondiabetics with FPG between 5.6mmol/L and 6.9 mmol/L (100 to 125 mg/dL) were classified as prediabetics, and individuals with a history of diabetes were classified as diabetics regardless of their FPG. Individuals with no history of diabetes but with FPG ≥7.0 mmol/L (126 mg/dL) were classified as diabetics for this study; in a clinical situation, most of these individuals would require a second FPG or a glucose tolerance test to confirm that assessment. Prevalence of diabetes and prediabetes in the group was compared to national prevalence using a Chi-square Goodness of Fit test, and differences were considered to be statistically significant when $p \leq 0.05$.

The categories of risk factors were: family history of a first-degree relative with diabetes, body mass index ≥25 kg/m^2, age ≥45, history of hypertension, HDL ≤0.91 mmol/L (35 mg/dL) or triglycerides ≥2.82 mmol/L (250 mg/dL), history of gestational diabetes, or birth of a baby over nine pounds. No assessment was made for physical inactivity. Risk factors were assigned a value of 1 if they were present and a value of 0 if they were absent. The total number of risk factors was summed for all participants. The PPV of a risk factor was calculated by dividing the total number of diabetics in each risk factor total category (true positives) by the total number of individuals in each category (true positives + false positives). The PPV of a risk factor for determining impaired glucose metabolism was calculated by dividing the total number of individuals with a FPG ≥5.6 mmol/L (100 mg/dL) and/or a diagnosis of diabetes in each risk factor total category (true positives) by the total number of individuals in each category (true positives plus false positives).

For the analysis of the euglycemics, all of the nondiabetics with FPG <5.6 mmol/L (100 mg/dL) were sorted by risk factor total category, and the means of the FPG were calculated for each category. Analysis of variance (ANOVA) was used to determine the probability that the difference in the means arose by chance, and was considered to be statistically significant if $p \leq 0.05$.

RESULTS

The participants were categorized as normal, prediabetic, or diabetic, and then sorted by risk factor total (Table 1). Forty-six individuals were classified as diabetics (6.9%); ten of these were unaware of their potential diabetic status. This compares well to the national prevalence of 6.3% ($\chi^2 = 0.610$, $p = 0.7371$).[4] All of the diabetics had at least one risk factor: <1% of the participants with one risk factor were diabetic, and this increased to 4.3% with two risk factors, 8.1% with three risk factors, 20.3% with four risk factors, and 54.5% with five risk factors. Only one participant had six risk factors, and this person was diabetic. The sensitivity of risk factor total to predict diabetes in this population was 100% for ≥ one risk, and dropped to 97.8% for ≥ two risks. Low values of risk factor total were nonspecific for diabetes in this population, and gave a high false positive rate. The PPV is a statistic that correlates the number of true positives with the number of false positives, and is a better indicator of the success of risk factor total in identifying diabetics. The formulae for calculating sensitivity, specificity, PPV and PPN are shown in Figure 1. The PPV of risk factor total for identifying diabetics is depicted in Figure 2 as closed squares.

The number of prediabetics was similar to the national prevalence as well: 135 of the participants (20.4%) fell into the newly defined range for prediabetes. Participants with one risk factor (8.9%) were prediabetic, increasing to 18.8% with two factors, 32.9% with three factors, 31.1% with four factors and 9.1% with five factors. The sensitivity of risk factor total to predict prediabetes was 100% for ≥ one risk, and dropped to 88.9% for ≥ two risks. The PPV of risk factor total for identifying individuals that have impaired glucose metabolism, that is those who are either prediabetic or diabetic, is depicted in Figure 2 as closed triangles.

The proportion of euglycemics in each risk factor group steadily decreased as the risk total increased, yet 49% of the participants with four risk factors and 36% with five risk factors were euglycemic. Analysis of variance was performed to examine the FPG mean for each risk factor total. Figure 3 shows the mean FPG of the euglycemics sorted by risk factor total. The lowest FPG was found for individuals with no risk factors (4.5 mmol/L or 83 mg/dL). The FPG mean increased with the risk total to a high of 5.2 mmol/L (95 mg/dL) in individuals with a five total risk factors ($p < 0.0001$).

Table 1. Risk factor total categories grouped by glycemic status

Risk factor total	0	1	2	3	4	5	6	Total
Normal	25	153	160	102	36	4	0	480
Prediabetic	0	15	39	57	23	1	0	135
Diabetic	0	1	9	14	15	6	1	46
Total	25	169	208	173	74	11	1	661

Figure 1. Formulae used for sensitivity and specificity

Sensitivity: % of the diabetics that are correctly identified by the criteria.

Sensitivity	TP/(TP + FN) * 100
Specificity	TN/(TN + FP) * 100
Sensitivity of ≥ 2 risk factors	45/(45 + 1) * 100 = **97.8%**

PPV: % of individuals in each category (risk factor total) that actually are diabetic.

Positive Predictive Value	TP/(TP + FP) * 100
Negative Predictive Value	TN/(TN + FN) * 100
PPV of 2 risk factors	9/(9 + 199) * 100 = **4.3%**

DISCUSSION

This Caucasian population approximated the national trends for diabetes and prediabetes, and showed that risk factor total is a sensitive method to predict individuals at risk for diabetes. The use of the risk total, however, showed a poor PPV for diabetes because it is nonspecific (Figure 2). For individuals with as many as four risk factors only 20% were diabetic, and for individuals with five risk factors 55% were diabetic. Risk factor total showed a better PPV for impaired glucose metabolism, that is either a FPG >5.5 mmol/L (99 mg/dL) or history of diabetes. The

PPV was lowest for a total of one factor (9.5%), and showed an almost linear increase up to 60% for individuals with five risk factors.

The American Diabetes Association recommends that individuals ≥45 years old be screened for diabetes every three years. Younger individuals should be considered for screening if they are obese or have any other 'risk factors'.[11] This leaves the decision to screen up to the physician. If the physician chose to screen all individuals with at least one risk factor, all of the diabetics in this study group would have been identified (100% sensitivity); that approach would have identified 100% of the subjects who were prediabetic as well. If, instead, the physician used the requirement of two or more risk factors before screening, he/she would miss 9.5% of the at-risk population.

Of the 25 individuals with no risk factors, all were euglycemic. This group had the lowest mean FPG, 4.5 mmol/L (83 mg/dL). It is interesting that the mean FPG increased as the risk factor total increased in the euglycemic subjects, and suggests that the individuals with a larger number of risk factors may be on the verge of becoming prediabetic. The recommendations for screening do support more frequent glucose testing in individuals who are high risk.[11] It appears from this study that more frequent screening of this group is indeed prudent.[11] It is possible that these individuals suffer from impaired glucose tolerance, and would be reclassified if they were tested by a two-hour glucose tolerance test.[7]

Figure 2. Positive predictive value of risk factor total

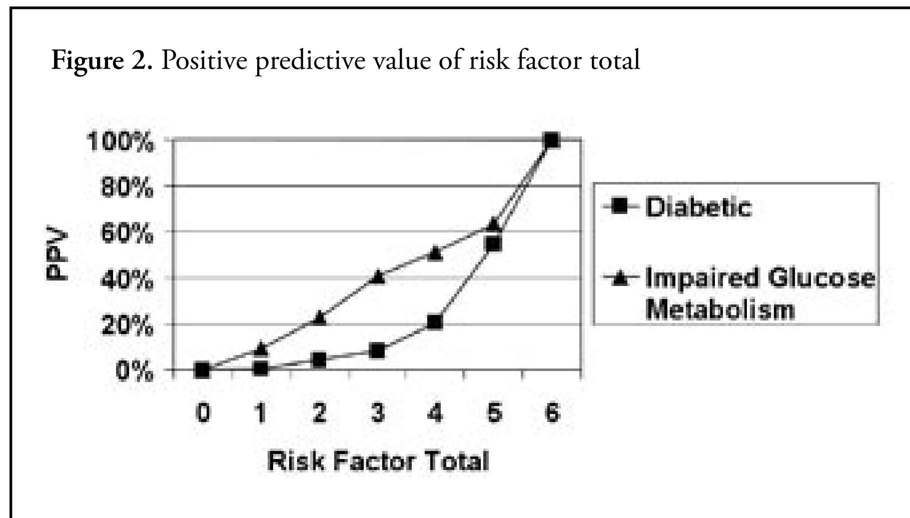
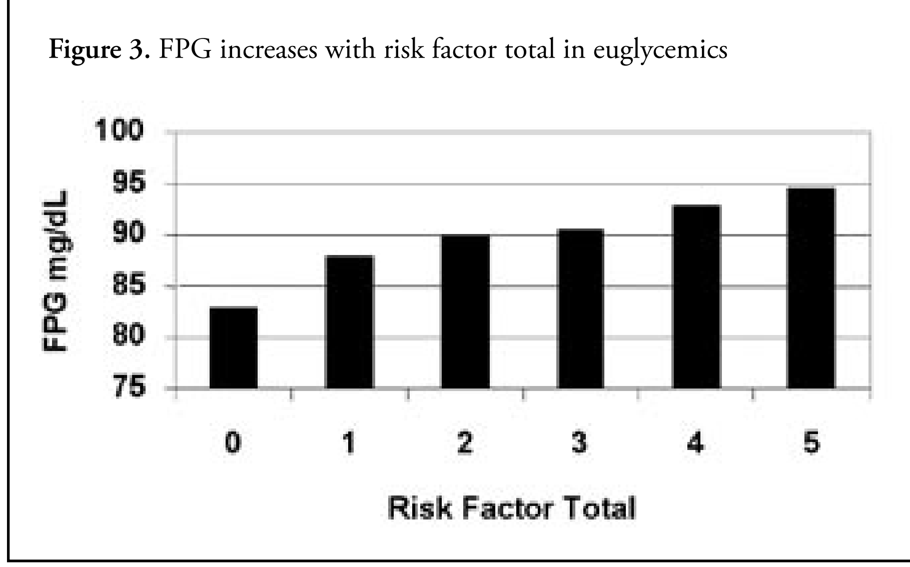


Figure 3. FPG increases with risk factor total in euglycemics



The American Diabetes Association Website offers a quiz to determine at-risk individuals, using weight, age, obesity, and physical activity as major predictors for diabetes.[4] The present study did not assess physical activity and treated all risk factors as equivalent, but it was nevertheless able to identify all subjects with impaired glucose metabolism. One limitation of this study is that there were relatively few subjects who had no risk factors. This study was performed on Caucasians only, and other race groups may show different results.

Many of the risk factors for diabetes are beyond the control of the individual, for example age, race, and family history. Other factors like hypertension, GDM, and dyslipidemias are in many instances linked to overweight and obesity. It is not surprising that there is a wave of increased diabetes in the U.S. that is concomitant with an increase in obesity.

Although the use of risk factor total showed a poor PPV for identifying diabetics in this study, it showed a good PPV for identifying individuals at risk for diabetes, both prediabetics and euglycemics. The current recommendation to screen all individuals for diabetes at age 45 or younger if they have one or more risk factors

appears to be appropriate.[11] Based on this study, screening individuals with one or more risk factors would result in 100% sensitivity for identifying individuals early who are at risk for the microvascular and macrovascular complications that are associated with hyperglycemia, and allow education, intervention and follow-up.

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REFERENCES

1. Winter W, Signorino MR. Diabetes mellitus, pathophysiology, etiologies, complications, management, and laboratory evaluation. Washington DC: AACC Press: 2002. p 51-63, 104-7.
2. American Heart Association. http://www.s2mw.com/heartofdiabetes/cardio.html. Accessed May 28, 2004.
3. Expert Panel on Detection and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
4. American Diabetes Organization. http://www.diabetes.org. Accessed December 8, 2003.
5. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2002;25:S5-S20.
6. Temelkova-Kurktschiev T, Koehler C, Leonhardt W, and others. Increased intimal-medial thickness in newly detected type 2 diabetes. Diabetes Care 1999;22:333-8.
7. The Expert Committee of the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160-7.
8. Saydah SH, Loria CM, Eberhardt MS, Brancati FL. Subclinical states of glucose intolerance and risk of death in the U.S. Diabetes Care 2001;24:447-53.
9. Coutino M, Gerstein H, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. Diabetes Care 1999;22:233-40.
10. American Diabetes Association and National Institute of Diabetes, Digestive and Kidney Diseases. The prevention or delay of type 2 diabetes. Diabetes Care 2002;25:742-9.
11. American Diabetes Association. Screening for Diabetes. Position Statement. Diabetes Care 2002;25:S21-4.

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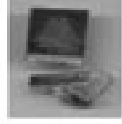

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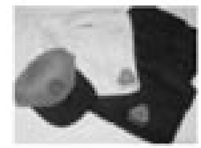

Students' Perceptions of Laboratory Science Careers: Changing Ideas with an Education Module

DANIEL HAUN, ARGIE LEACH, LOUANN LAWRENCE, PATSY JARREAU

OBJECTIVE: To assess the effectiveness of a Web-based education module in changing students' perceptions of laboratory science careers.

DESIGN: Perception was measured with a short examination and then a Web-based exercise was presented. Following the exercise, the test was administered again. Frequency data from the pre-test and post-test were compared for changes in perception. The correlated pre-test/post-test pairs were also examined for opinion changes and these were analyzed for significance.

SETTING: Large parochial high schools in New Orleans, Louisiana. A small team visited the schools during their appointed class times for biology.

PARTICIPANTS: Study participants were high school biology students in grades 9–10. Two-hundred-forty-five students participated (149 male and 96 female).

INTERVENTIONS: A Web-based exercise on blood film examination was presented to the students in a classroom setting (www.mclno.org/labpartners/index_03.htm). The exercise contained focused messages about: 1) the numbers of healthcare workers acquiring AIDS from on-the-job exposure and 2) common career paths available to the laboratory science workforce.

MAIN OUTCOME MEASURES: The shift in perception of:

- what medical service generates the most diagnostic data
- which professional group performs laboratory tests
- the risk of acquiring AIDS while working in the healthcare setting
- interest in a science-related career
- how much education is required to work in a science-related field

RESULTS: The intervention significantly shifted perception in all areas measured except that of interest in a science-related career.

CONCLUSIONS: Many students perceive that the risk of acquiring AIDS while working in the healthcare setting is "high". Web-based presentations and similar partnerships with science teachers can change perceptions that might lead to increased interest in clinical laboratory science careers.

ABBREVIATIONS: CCCLW = Coordinating Council on the Clinical Laboratory Workforce; CLS = clinical laboratory scientist; CLT = clinical laboratory technician.

INDEX TERMS: community outreach; community-institutional relations; career choice education; Internet education.

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Recruitment of qualified applicants is a high priority for clinical laboratory science (CLS) and clinical laboratory technician (CLT) educational programs in Louisiana and in most areas of the nation. Currently there is a national shortage of qualified laboratory personnel as evidenced by a current national vacancy rate of 7.0% for CLSs and 8.6% for CLTs.[1] Based on demographic information from the ASCP member database, more than 72% of the current laboratory workforce is older than 40 years of age.[1] It is predicted that attrition in the profession due to retirement will decrease the workforce dramatically during the next 10 to 20 years. The demand for certified CLSs

and CLTs will continue to increase as the nation ages and the proportion of elderly needing healthcare continues to increase.[2] The number of CLS programs in the nation has decreased 62% during the last 19 years.[3] The increasing demand and decreasing supply of qualified laboratorians make recruitment a problem that requires intensive effort.[4]

One of the reasons for lack of qualified applicants is lack of knowledge of the profession as a possible career path. It is often said that CLS is the "hidden profession". The Coordinating Council on the Clinical Laboratory Workforce (CCCLW) is comprised of representatives from various laboratory professional organizations, industry groups, and government agencies. It was established to develop a strategic plan to address the shortage of clinical laboratory personnel. One of the objectives of the CCCLW is to increase the awareness of the profession as a career choice by designing projects to facilitate a relationship with K–12 school systems, high school guidance counselors, and science teachers.[5]

In 2000 and 2001, two summit meetings of the CCCLW were held. In Summit I (June 2000), four breakout groups identified the "components of the problem". All four groups cited image and public recognition as components and three of four groups cited "danger" as a component of the problem, using the words "danger", "dangerous", and "dangerous working conditions" as component descriptors.[6] The report for Summit II cites the "risk of infectious disease" as one of the seemingly recognized reasons for instability in the clinical laboratory labor pool supply.[7]

The project described in this paper was designed to reveal the profession to prospective students by providing resources, lesson plans, and support from local laboratorians for middle school and high school science teachers. By giving teachers a resource that is easily accessible and providing an incentive for use in their classrooms, more students will be introduced to CLSs/CLTs earlier in their education. Many middle school and high school students who think they may be interested in a health career only know about doctors and nurses. This Web-based approach was also designed to dispel the myths that it is dangerous to work in a laboratory and that one must have many years of education to have a meaningful career in science and medicine. The Web-based nature of this resource makes it instantly available to science teachers and CLS/CLT educational programs nationwide.

PURPOSE

The purpose of the study is to assess the effectiveness of a Web-based education module in changing the students' perceptions. A pre-test and post-test were used to measure perception and to assess ideas for changing the perceptions of high school students about CLS careers. The tests sought to measure the student's awareness of the laboratory workforce, the perceived danger of contracting HIV while working in the healthcare setting, and general interest in and knowledge of science-based careers.

METHOD

A team of two technologists visited biology classrooms with a computer-based exercise on blood film examination. The entire exercise was conducted during the normal class period in a 50-minute time frame. A pre-test was administered consisting of six multiple-choice questions and four statements with Likert scales to assess perception. The exercise was then conducted and a post-test was administered. The post-test frequency data was then compared to the pre-test frequency data to assess the effectiveness of the interventions. The correlated (paired pre- and post-test) data were also examined to quantitate number of students who changed their perceptions following the intervention.

TEST DESIGN

The pre-test and post-test were identical in content and began with five multiple-choice questions covering the topical substance of the exercise. These five questions were not graded for the purpose of the study but were included to enhance the interest and participation of the students. To measure the perception of image and public recognition, we designed one multiple-choice question and one Likert scale item (Figure 1, numbers 6 and 7). To measure the perception that healthcare careers were dangerous, we used a Likert scale item on the probability of acquiring HIV/AIDS (Figure 1, number 8). We chose this because preliminary interviews with students indicated that HIV could be a feared consequence of working in healthcare. One Likert scale item asked about interests in science-related careers (number 9) and finally one Likert scale item addressed the availability of science-related careers without an advanced degree (number 10).

SAMPLE

Teams presented the intervention to eleven general biology classes at large parochial high schools, grades 9 and 10. A total of 245 students were surveyed (149 males and 96 females). The schools were chosen because of high teacher interest, which facilitated scheduling.

MATERIALS

The intervention consisted of a Web-based module that was delivered locally from a notebook computer and projected

to the class using a liquid crystal display projector.[8] The module contains many elements and teacher resources, e.g., image galleries and science project ideas, but only four elements (listed below) were presented to the class.

1. An overview presentation of the complete blood count and WBC differential – 10 minutes.
2. A short presentation on the risk of acquiring HIV in the healthcare setting – 2 minutes.
3. A short presentation on the career path for CLSs and CLTs – 2 minutes.
4. A blood film examination exercise that simulated a twenty-five cell differential on two patients – 35 minutes.

PROCEDURE

The team was introduced by name and university affiliation to the class by the teacher, and the team immediately distributed the pre-test. After approximately five minutes, the pre-tests were collected. One team member began the presentation with the introduction to the CBC and blood film examination. Next, the presenter initiated the blood film exercise on the first 'patient'. As the exercise was started, it was interrupted with a short 'commercial' explaining the risk of acquiring HIV while working in the healthcare setting (Figure 2). The exercise required students to identify common white blood cells, calculate percentages, and evaluate the results by comparison with reference ranges. Finally, the students were to determine the likely diagnosis from the numerical data using information presented in the introduction.

Then the presenter started the second 'patient' presentation that contained a second 'commercial' on the career paths for laboratory science (Figure 3). Career paths for technologist and technician were discussed along with salary expectations. 'Patient two' was then evaluated in the same manner as patient one. The class worked as a team with members calling out cell types and the presenters settling disputes by using the embedded help screens.

Figure 1. Student assessment - blood film examination exercise.

Pretest

6. Which statement best fits your idea of how medical diagnoses are made___
a. Most diagnostic data is generated by the doctors.
b. Most diagnostic data is generated by nurses.
c. Most diagnostic data is generated by the radiology (XRAY) department.
d. Most diagnostic data is generated by the lab.

For the statements below, circle the choice that best-fits your opinion.

7. Most blood tests are performed by doctors and nurses

Strongly agree	agree	No opinion	disagree	Strongly Disagree

8. I would rate the danger of catching AIDS in a health care career as extremely low.

Strongly agree	agree	No opinion	disagree	Strongly Disagree

9. I am very interested in a science-related career.

Strongly agree	agree	No opinion	disagree	Strongly Disagree

10. Most science careers require an advanced doctorate degree (for instance PhD. or M.D.)

Strongly agree	agree	No opinion	disagree	Strongly Disagree

RESULTS

Data are presented and discussed in two sets:

1. Frequencies of all responses expressed in percent: the pretest frequency data is presented in Table 1 and post-test frequency data is presented in Table 2. Only questions with answers were tallied for the frequency data and the number tallied varies by question.
2. Correlated data, expressed in number of students: individual pre and post responses are compared and evaluated using the McNemar test for correlated samples to assess the significance of the change in opinion.[9] These results are displayed in Table 3. Students who answered in one test but not in the other test were not included in the McNemar test. For the correlated data, values of $p < 0.05$ are considered to be significant.

Responses to specific items

Most diagnostic data is generated by the laboratory.

The frequency analysis showed that in the pre-test, 65% of students rec-

ognized that the laboratory generated the majority of diagnostic data and this increased to 90% in the post-test. The correlated data analysis showed that seventy students changed their perception toward the laboratory and only three changed in favor of other disciplines, which is a significant change. For the correlated analysis, eleven students did not answer the question, either in the pre-test or post-test and were excluded.

Most blood tests are performed by doctors and nurses.
The frequency analysis showed that initially 71% of students indicated that blood tests are performed by doctors and nurses but after the exercise this dropped to 33%. In the correlated analysis, 117 students changed their mind toward the laboratory position and only 11 changed toward the doctors and nurses, which is a significant change. For the correlated analysis, one student did not answer the question in the pre-test and was excluded.

I would rate the danger of catching AIDS in a healthcare career as extremely low.
Frequency analysis indicated that, before the exercise, only 29% agreed that the chance of catching AIDS in a healthcare career was 'extremely low' but after the exercise, this perception increased to 77%. The correlated analysis showed that 137 students changed their minds from 'disagree', while only 17 changed from 'agree', which is a significant change.

I am very interested in a science-related career.
Frequency analysis showed that, in the pre-test 57% of students indicated a high interest in a science-related career and this increased slightly to 60% post-intervention. The correlated data showed that 34 changed their mind from the 'disagree' position while 21 changed from the 'agree' opinion and this change is insignificant.

Most science careers require an advanced doctorate degree, e.g., PhD or MD.
Frequency data reveal that 72% agreed before the exercise; this decreased to 55% after the session. Most students in the post-test still thought that an advanced degree was necessary. The correlated analysis showed that 78 students changed their mind from the 'agree' position while only 22 changed from the 'disagree' position, which is significant.

Figure 2.

Is lab work dangerous because of HIV & AIDS?

Yes No.

Many people think so, but since 1978 there have been a total of 46 health care workers who have caught AIDS from on-the-job exposure.

Sixteen of these were laboratory workers and 23 were nurses

There have been no new infections in the past several years.

We now use protective gloves and other safety devices to prevent exposure. The risk of infection is very low.

Figure 3.

First a short commercial.

Then start.

Laboratory professionals can practice as either:

Medical Laboratory Technicians- *2 yr. associate degree*

or as

Medical Technologists (Clinical Laboratory Scientists) these have bachelor of science degrees

Technologists make more money and have increased responsibilities for lab quality and management.

Both degree curriculums are special. They teach topics and skills specific to the understanding and measurement of human biology. If you want to practice laboratory medicine, you must enroll in one of these special programs. You can find out more under the career information section.

DISCUSSION

The data suggest that while most students understand that the laboratory provides diagnostic data, they mostly perceive that laboratory tests are performed by doctors and nurses. The data further suggest that the intervention

Table 1. Pretest frequency data for all students in percentage of responses to each available choice

	Doctors	Nurses	Radiology	Laboratory	N*
Most diagnostic data is generated by the	17.5%	6.3%	9.6%	66.7%	240

	Strongly agree	Agree	No opinion	Disagree	Strongly disagree	N*
Most blood tests are performed by doctors and nurses	20.6%	52.3%	8.2%	16.5%	2.5%	243
I would rate the danger of catching AIDS in a healthcare career as extremely low	7.0%	21.9%	19.8%	38.4%	12.8%	242
I am very interested in a science-related career	21.2%	36.9%	22.0%	13.3%	6.6%	241
Most science careers require an advanced doctorate degree, e.g., PhD or MD	20.7%	53.5%	13.3%	10.8%	1.7%	241

* N varies by question because some students did not answer all questions

Table 2. Post-test frequency data for all students in percentage of responses to each available choice

	Doctors	Nurses	Radiology	Laboratory	N*
Most diagnostic data is generated by the	1.7%	2.9%	2.9%	92.5%	239

	Strongly agree	Agree	No opinion	Disagree	Strongly disagree	N*
Most blood tests are performed by doctors and nurses	10.2%	22.9%	7.3%	35.5%	24.1%	245
I would rate the danger of catching AIDS in a healthcare career as extremely low	42.9%	34.3%	7.3%	9.4%	6.1%	245
I am very interested in a science-related career	22.0%	38.4%	24.1%	9.8%	5.7%	245
Most science careers require an advanced doctorate degree, e.g., PhD or MD	13.1%	42.2%	13.9%	27.5%	3.3%	244

* N varies by question because some students did not answer all questions

both increased the awareness of the clinical laboratory and largely recalibrated the myth that doctors and nurses perform the 'blood tests'.

In the pre-test, a majority of students disagreed with the statement that the risk of catching AIDS in a healthcare career was 'extremely low'. This suggests that the perceived dangers of contracting an infectious disease could be a significant deterrent to choosing a career in healthcare. The short 'commercial' which simply presented the data on occupationally acquired AIDS cases was highly effective in changing this perception. The data suggest that all healthcare career information should contain the facts on workplace safety.

The exercise seemingly did not increase interest in science-related careers. This increase was hoped for and predicted by teachers on the module design team. The reasoning was that the exercise would demonstrate a practical science-based occupation, where one could practice without an advanced degree. The exercise did change ideas about the necessity of an advanced degree, which might increase interest in the field. However, a majority of students continued to believe that an advanced degree was necessary for a science-based career.

CONCLUSION

The two CCCLW meetings on the shortage of clinical laboratory personnel identified many important components of the problem and the workgroups offered an array of opportunities to intervene. Among the opportunities suggested was a Web-based education module for K–12 educators to improve the image and awareness of the profession. This study demonstrates that such a module could be effective in increasing awareness in high school students and in recalibrating false perceptions and fears that might discourage potential candidates. The study is limited because the module was not delivered via the Internet but was done in a live presentation to facilitate data collection. There is little doubt that the presenters added interest and emphasis that is otherwise absent when a student visits a Web page. However, as this Web-based exercise is available to laboratorians and educators alike, we offer that a grass-roots effort based on local partnerships (laboratorians working with science teachers) may significantly increase the number of students interested in laboratory careers.

The effect of the laboratorian/teachers partnership was not measured by this study but both groups reported a high level of satisfaction with the experience. Ideas for future collaborations are being discussed and a new project to help teachers obtain low cost digital microscopes has already been initiated.[10] Others seeking to mimic this study could take advantage of an updated and expanded version of the Website at http://www.mclno.org/labpartners/index.htm.

Table 3. Correlated test data for individual changes in opinion

	Changed opinion in favor of statement	Changed opinion against statement	N	Did not answer*	*p*
Most diagnostic data is generated by the lab	70	3	234	11	<0.0001
Most blood test are performed by doctors and nurses	11	117	244	1	<0.0001
I would rate the danger of catching AIDS in a healthcare career as extremely low	137	17	245	0	<0.0001
I am very interested in a science-related career	34	21	245	0	0.1056
Most science careers require an advanced doctorate degree, e.g., PhD or MD.	22	78	245	0	<0.0001

* Answered in one test but not in the other. Those who did not answer in both tests were counted as not changing answer (opinion).

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REFERENCES

1. Ward-Cook K, Chapman S, Tannar S. 2002 Wage and vacancy survey of medical laboratories. Lab Med 2003;34(10):702-7.
2. U.S. Department of Labor, Bureau of Labor Statistics. Occupational Outlook Handbook. 2002–03 Ed. http://www.bls.gov. Accessed May 10, 2003.
3. National Accrediting Agency for Clinical Laboratory Sciences (NAACLS); Chicago: Annual Report 2002.
4. Ward-Cook K, Daniels MG, Gueroguieva J. ASCP Board of Registry's 2001 Annual Survey of Medical Laboratory Science Programs. Lab Med 2002;33(11):831-6.
5. Coordinating Council on the Clinical Laboratory Workforce, The U.S. clinical laboratory workforce: strategic plan. ASCP; Chicago: July 1, 2002.
6. Summit on the Shortage of Clinical Laboratory Personnel, Summit I. http://www.ascls.org/ssclp/ssclp1_4.asp. Accessed 6/3/2004.
7. Summit on the Shortage of Clinical Laboratory Personnel, Summit II. http://www.ascls.org/ssclp/ssclp2.asp. Accessed 6/3/2004.
8. Your hospital laboratory. http://www.mclno.org/labpartners/index_03.htm. Accessed 7/18/04.
9. Portney LG, Watkins MP. Foundations of clinical research, applications to practice. McGraw Hill Appleton and Lange; Columbus OH: 1993. p 498-9.
10. Build your own digital microscope. www.mclno.org/labpartners/microscope/index.htm. Accessed July 25, 2005.

POSITION ADVERTISEMENT

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Applicants must have: 1) an earned doctorate and be certified as an allied health professional in one of the areas offered by the programs in the College; 2) teaching, research, and scholarly experience; 3) substantive academic administrative experience at the departmental level or higher; 4) demonstrated ability to interact and negotiate successfully with both internal and external constituencies; 5) an understanding of the academic and clinical allied health sciences, particularly as they relate to broader health care issues; and 6) a forward-looking vision of the allied health sciences within the university, community, and state. Experience with successful external funding activities and faculty practice plans preferred.

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The Reemergence of Pertussis in Immunized Populations: A Case Study

DELFINA DOMÍNGUEZ

OBJECTIVE: To present a case of classical pertussis occurring in previously vaccinated male siblings, 11 and 13 years of age, living in El Paso TX; also to present an overview and update of the changing epidemiology of pertussis including pathophysiology, diagnosis, and treatment.

DESIGN: Nasopharyngeal swabs and blood samples were collected from two male siblings, 11 and 13 years of age, presenting with cold-like symptoms and persistent cough during the second week of infection. Nasopharyngeal swabs were plated onto Bordet-Gengou agar plates and incubated for 48 hours. Blood samples were analyzed for the presence of antibodies (IgM and IgA) against *Bordetella pertussis* antigens using indirect enzyme linked immunosorbent assay (ELISA).

SETTING: Cultures and serological analysis was conducted at the University of Texas at El Paso, Clinical Laboratory Science Program Research facility.

RESULTS: Bacterial cultures of both children were positive for *Bordetella pertussis* and the sera revealed positive IgM and IgA antibodies (>11 PANBIO UNITS) against a mixture of antigens including: pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae.

CONCLUSIONS: Pertussis immunity wanes overtime, leaving most adolescents and adults susceptible to infection. Physicians must be prepared to diagnose and treat pertussis in any age group regardless of vaccination status.

ABBREVIATIONS: AC = adenylate cyclase; DaTP = acellular DTP; DFA = direct fluorescent antibody; FHA = filamentous hemaglutinin; PCR = polymerase chain reaction; PRN = pertactin; UTEP = University of Texas at El Paso.

INDEX TERMS: *Bordetella pertussis*; pertussis epidemiology; pertussis.

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Bordetella pertussis, the etiologic agent of whooping cough, continues to be an important cause of morbidity in the United States.[1-3] Despite vaccination coverage for over 50 years, pertussis has not been eradicated.[3-6] Primary vaccination is effective in 80% of the cases.[7] However, this protection is transient making the vaccinated host vulnerable to infection.[7-9] Recently, major outbreaks of the disease have been documented in highly immunized populations worldwide and throughout the U.S.[6,10-17] Data from these outbreaks reveal a remarkable epidemiological shift. Historically, the occurrence of pertussis was primarily in infants or under-immunized pre-schoolers. These recent studies now demonstrate an epidemiological shift of increasing disease in older children, adolescents, and adults.[5,6,18,19] The cause of the increased incidence has not been definitively determined. However, factors such as waning vaccine-induced immunity, vaccine quality, antigenic variation of *Bordetella* strains, and undetected mild illness in adults and adolescents have been proposed.[18,20] Outbreak investigations have documented that *B. pertussis* infections occurring in adults have subsequently been transmitted to other adults and children.[8,9] Therefore, previously immunized adults and adolescents are an important reservoir for *B. pertussis.*[16,19,21] Pertussis can be difficult to diagnose and is often unrecognized or misdiagnosed in adolescents and adults, since the classic presentation of whooping cough may not be present. In addition, laboratory results may be non-diagnostic if the specimen is not appropriately collected.[7,22,23] The following

The peer-reviewed Research and Reports Section seeks to publish reports of original research related to the clinical laboratory or one or more subspecialties, as well as information on important clinical laboratory-related topics such as technological, clinical, and experimental advances and innovations. Literature reviews are also included. Direct all inquiries to David G Fowler PhD CLS(NCA), Clin Lab Sci Research and Reports Editor, Dept of Clinical Laboratory Sciences, University of Mississippi Medical Center, 2500 North State St, Jackson MS 39216. (601) 984-6309, (601) 815-1717 (fax). dfowler@shrp.umsmed.edu

is a case of classical pertussis occurring in El Paso TX in male siblings despite vaccination coverage.

CASE PRESENTATION

Two Caucasian male siblings, ages 11 and 13 years, each with a history of asthma, presented with cold-like symptoms and a light persistent cough of 10-day duration. Their pediatrician diagnosed a viral infection with no apparent signs of a serious illness. The patients were treated with cough syrup and decongestants. A couple of weeks later, the boys developed progressive coughing spells with inspiratory whoop and post-tussive vomiting. The boys re-visited their pediatrician's office, going home without a specific diagnosis or treatment plan, though it was apparent that their condition had worsened considerably. When the mother inquired about the possibility of a pertussis infection, the answer was that the boys were fully vaccinated and such infection was very unlikely. The anguished mother heard about the UTEP *Bordetella pertussis* research and contacted the investigator. Nasopharyngeal swabs were collected at this time (during the second week of infection) and blood samples were collected for serology. The cultures of both children were positive for *Bordetella pertussis* and the sera revealed positive IgM and IgA antibodies (>11 PANBIO UNITS) against a mixture of antigens including: pertussis toxin, filamentous hemaglutinin, pertactin, and fimbriae assayed by indirect enzyme linked immunosorbent assay (ELISA) (IgM, IgA ELISA kits, Panbio, Australia).

The mother took the children to a pediatric infectious disease specialist, who made the diagnosis of pertussis. The 13-year-old child had received the complete DPT series (five DPT doses) while the 11-year-old boy received only three DPT doses due to a prior reaction. The two boys and other family members were placed on a course of azithromycin. Both boys showed clinical improvement thereafter, but persisted with a cough for several weeks.

DISCUSSION

In this era of antibiotics and high vaccination coverage, pertussis has been relegated from a prime childhood killer to a half-forgotten threat. However, during the past few years, evidence shows that pertussis continues to be a threat for North American children and the adult population.[1,2,24] Pertussis vaccines have been effective in controlling infant disease, but the transmission of *Bordetella pertussis* has not been eliminated.[21,26] Vaccine- induced immunity wanes after five to ten years, allowing vaccinated hosts to become susceptible to infection.[8,26] This susceptibility is reflected in recent outbreaks in highly vaccinated populations worldwide.[9-14]

For the past few years, pertussis has been a health concern in Texas.[17] Since March 26, 2000, 14 deaths have been reported, and several outbreaks occurred throughout the state, and more than 200 hospitalizations have been reported in 2002.[27,28]

The present case report highlights the need for awareness and education of pertussis infections to our communities and for clinicians to consider the diagnosis of pertussis in older children and adults, despite either an atypical clinical presentation or lack of laboratory confirmation. More important, clinicians should not exclude the diagnosis of pertussis based on vaccination status.

PATHOGENESIS AND PATHOPHYSIOLOGY

Pertussis is a highly contagious respiratory disease that is caused by the gram-negative, coccobacillus *Bordetella pertussis*. Humans are the only reservoir. No animal or vector is known to exist. Transmission occurs through contact with airborne droplets of respiratory secretions. The contagious period is seven days following exposure to the organism and during the catarrhal stage. This period is usually two to three weeks after the onset of symptoms. Pertussis lacks a seasonal pattern, however, most cases occur during the summer and fall.[3,29] *B. pertussis* produces several virulence factors that are involved in the pathogenesis of the disease. The following pathophysiological sequence of events has been proposed by Weiss and Hewlett: 1) attachment, 2) evasion of host defenses, 3) local damage, and 4) systemic effects.[30] Two major non-fimbrial adhesions play an important role in attachment: filamentous hemaglutinin (FHA) and pertactin (PRN). Mutants lacking either one are unable to attach ciliated epithelial cells and cause ciliostasis and cell damage.[31-33] Pertactin stimulates leukocytes to express integrins, which function as receptors for FHA, leading to macrophage phagocytosis via CR3 avoiding oxidative burst and triggering intracellular survival.[32,34] Following attachment and onset of infection, adenylate cyclase (AC) toxin plays an important role in cell damage and impaired leukocyte function. The cytotoxic activity of the AC results when the enzyme is activated by the host calmodulin leading to uncontrolled production of cAMP altering the metabolism of the invaded cell.[35] Tracheal cytotoxin and possibly dermonecrotic toxin are involved in local damage to ciliated cells. It is believed that systemic disease is mediated by pertussis toxin. Despite the identification and purification of a number of antigens and active components of *B. pertussis* the pathogenesis of infection is poorly understood and the immunity of infection is incompletely defined.

CLINICAL CHARACTERISTICS

Pertussis signs and symptoms manifest after an incubation period of one to two weeks. Typical pertussis has been divided into three clinical phases: catarrhal, paroxysmal, and convalescent. During the catarrhal phase, mild upper respiratory symptoms with progressive cough occur. This phase lasts one to two weeks. Due to the minimal symptoms during this phase, the diagnosis of pertussis is rarely considered. However, the recovery of the organism is the highest at this time.[29,32] The paroxysmal phase of pertussis is characterized by severe cough classically followed by an inspiratory whoop. Post-tussive vomiting is common and cough paroxysms are worst at night. However, only 6% of the patients exhibit the classic inspiratory whoop.[3] Most complications of pertussis occur during this phase, including secondary infections (otitis media and pneumonia), trauma associated with paroxysmal cough, and central nervous system abnormalities.[36] This paroxysmal phase lasts four to six weeks and is the period in which pertussis is easily recognized. During the convalescent or recovery phase there is a gradual decrease of cough until it disappears completely. This phase lasts several weeks. In older persons, adults and adolescents, or those partially protected by the vaccine the manifestation of the disease is atypical. Pertussis may present as persistent cough (>six days) and may be indistinguishable from other upper respiratory infections. Some studies have reported isolation of *B. pertussis* from 25% or more of adults with cough illness lasting >seven days.[33,36,37]

DIAGNOSIS

Confirmation of *B. pertussis* infection can be one of the most difficult challenges the clinician can face. The gold standard and preferred laboratory test is isolation of the organism by culture.[38] Isolation of *B. pertussis* is readily achieved during the catarrhal phase, however it is during this phase that the diagnosis of pertussis is often not suspected, especially in older children and adults.[33] Specimens from the nasopharynx, not the throat, should be obtained using a calcium alginate or Dacron™ swab. Cotton swabs should not be used because these contain fatty acids, which inhibit bacterial growth.

Bordetella pertussis is a very fastidious, slow-growing organism and the use of selective media (Bordet Gengou or Regan-Lowe agar) is required to plate the specimen. Success in isolating the organism declines with prior antibiotic therapy (erythromycin or trimethoprim-sulfamethoxazole) or delay in collecting the specimen. Culture is 80% sensitive when the specimen is collected during the first two weeks of infection. Sensitivity falls to 15% after the fourth week and 0% after five weeks of infection.[39,40] Nasopharyngeal specimens can also be valuable for the identification of pertussis by direct fluorescent antibody technique (DFA). The advantage this method offers is that the organisms do not have to be viable for detection, and therefore can be detected later in the course of infection. In addition, DFA can be used in the presence of antibiotics. However, DFA should not be used as a sole criterion for laboratory confirmation since various studies have shown low sensitivity and variable specificity.[29,32,39] Pertussis serology has been useful in clinical studies. It is available in some reference microbiology laboratories but is not yet standardized. Due the lack of reference values in healthy individuals and to the lack of association between antibody levels and immunity, results of serologic tests are difficult to interpret. Cases meeting the clinical case definition and serological positive but culture negative should be reported as probable cases.[29,41,42] The polymerase chain reaction (PCR) performed on nasopharyngeal material or aspirates has been found to be a rapid, sensitive, and specific method of diagnosis. Polymerase chain reaction tests should be validated and always should be offered in addition to culture but never as a replacement for culture since bacterial isolates are required for evaluation of antimicrobial resistance and for molecular typing.

In contrast to infants, immunized children and adults may not show the typical symptoms of pertussis. More typically, these patients may exhibit an insidious onset and symptoms of a mild, nonspecific upper respiratory tract infection. Pertussis in adults is not well characterized but usually consists of a prolonged cough that may last for weeks or months.[9,21,26,43] Physicians may not recognize pertussis in older children and adults who do not exhibit the classical illness. In turn, untreated children and adults may serve as a reservoir to susceptible infants.[8,44] In 1999, approximately 10% of pertussis cases reported in the U.S. occurred among persons 15 years of age or older, and outbreaks of pertussis among adults and adolescents have been reported worldwide.[31-34] Several reports indicate that 10% to 25% of patients with cough lasting two weeks or longer have been due to pertussis.[5,45-48] Epidemiological data is limited, but it has been estimated that the incidence of pertussis may be in the range of 170 to 500 cases per 100,000 adults.[9,45] Although pertussis in adults varies from subclinical infection to persistent cough, the morbidity related with pertussis in adults increases with age. Complications may include pneumonia, rib fracture, hernia, and otitis media.[49] At present, data from outbreaks, clinical and seroprevalence studies, and surveillance data suggest that adults and adolescents may be a reservoir for *B. pertussis* infection.[9,17,24,50-54]

In summary, pertussis diagnosis is based on clinical history with supportive laboratory data. Currently no single test offers acceptable sensitivity and specificity in the diagnosis of pertussis. Laboratory diagnosis is limited due to the incomplete understanding of pertussis immunity and poorly understood pathogenesis of infection.

TREATMENT AND PREVENTION

Erythromycin has been the drug of choice (14-day treatment), but azithromycin has been an effective replacement and is given for five to seven days. Antibiotic therapy eliminates the organism from secretions thus decreasing communicability and shortening the course of illness if administered early. Trimethoprim-sulfamethoxazole is an effective alternative in patients who cannot tolerate erythromycin. Prophylaxis with either drug should be administered for 14 days to all household contacts and other close contacts of persons with pertussis regardless of age and vaccination status. Management of pertussis during an outbreak requires early recognition and treatment of infected persons and contacts, complete immunization of all susceptible children, and case reporting to public health authorities. Currently, acellular vaccine (DTaP) is recommended for all doses of pertussis schedule. Whole-cell vaccine is no longer recommended in the U.S. The complete series of DTaP consists of four doses given at four to eight week intervals except for the fourth dose, which is given six to twelve months after the third dose in order to maintain adequate immunity for the preschool years.[29,41] A new booster vaccine for adults and adolescents has been approved recently. This new vaccine has the same components (diphtheria and tetanus toxoids and acellular pertussis antigens) but it is formulated with reduced antigen quantity. The new vaccine is indicated for active booster immunization as a single dose in individuals 10 through 18 years of age.

CONCLUSIONS

Introduction of the whole cell pertussis vaccine in 1949 led to the dramatic decline of pertussis and related complications. Nevertheless, the number of pertussis cases has increased steadily during last few years. The growth of an infected adult and adolescent population appears to be the cause of the recent reemergence of pertussis in the U.S. However, the possibility of antigenic variation in circulating strains of *B. pertussis* has not been studied. An optimal control of pertussis could be achieved through the immunization and periodic booster vaccines to adult and adolescent populations. The availability of this new booster vaccine will provide an opportunity to reduce disease incidence and circulation of *B. pertussis* in our communities.

In summary this case highlights the following points:

1. Pertussis is not a disease that occurs just among children. Studies show that children and young infants acquire the infection from parents, grandparents, or other older adults.
2. Physicians should be aware that vaccine immunity to pertussis wanes with time, and that pertussis may occur in any age group regardless of vaccination status.
3. Because of inherent difficulties in the laboratory diagnosis of pertussis, physicians must be prepared to diagnose and treat pertussis clinically in any age group.
4. A greater awareness and more aggressive approach to childhood and adult pertussis could be instrumental in decreasing the risk and ultimately the incidence of infant pertussis.

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REFERENCES

1. Teitelbaum MA. Immunization and vaccine-preventable illness, United States, 1992-1997. Stat Bull 1999:13-20.
2. Deeks S, De Serres G, Boulianne N, and others. Failure of physicians to consider the diagnosis of pertussis in children. Clin Infect Dis 1999;28:840-6.
3. Guris D, Strebel PM, Bardenheier B, and others. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. Clin Infect Dis 1999;28:1230-7.
4. Black S. (1997). Epidemiology of pertussis. Pediatr Infect Dis 1997;16(Supplement):S85-9.
5. Cromer BA, Goydos J, Hackell J, and others. Unrecognized pertussis infection in adolescents. AJDC 1993;147:575-7.
6. Deville JG, Cherry JD, Christenson PD, and others. Frequency of unrecognized *Bordetella pertussis* infections in adults. Clin Infect Dis 1995;21:639-42.
7. Scott PT, Clark JB, Miser WF. Pertussis: an update on primary prevention and outbreak control. Amer Fam Phys 1997;56(4).
8. Srugo I, Benilevi D, Madeb R, and others. Pertussis infection in fully vaccinated children in day-care centers, Israel. CDC 2000; 6(5).
9. Strebel P, Nordin J, Edwards K, and others. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995-1996. J Infect Dis 2001;183:1353-9.
10. Al-Bargish KA. Outbreak of pertussis in Basra, Iraq. East Mediterr Health J 1999;5(3):540-8.
11. De Melker HE, Conyn-van Spaendonck MAE, Rumke HC, and others. Pertussis in the Netherlands: an outbreak despite high levels of immunization with whole vaccine. Emerg Infect Dis 1997;3:175-8.
12. Moi FR, Van Oirschot H, Heuvelman K, and others. Polymorphisms in the *Bordetella pertussis* virulence factors P 69/pertactin and pertussis toxin in the Netherlands: temporal trend and evidence for vaccine-driven evolution. Infect Imm 1998;66:670-5.

13. Moi FR, He Q, Van Oirschot H, and others. Variation in the *Bordetella* virulence factors pertussis toxin and pertactin in vaccine strains and clinical isolates in Finland. Infect Imm 1999;67:3133-4.
14. Prevost G, Stoessel FIS, Meunier O, and others. Analysis with a combination of macrorestriction endonucleases revelas a high degree of polymorphism among *Bordetella pertussis* isolates in eastern France. J Clin Microbiol 1999;37:1062-8.
15. Prevention, Centers for Disease Control. Pertussis outbreak-Vermont 1996. MMWR 1997;46:822-6.
16. Prevention, Centers for Disease Control. Whooping cough. National Center for Disease Statistics 2000.
17. Pelosi JW, Tulu A, Tabony L. Pertussis in Texas. TDH – DPN 2001;61(16).
18. Heininger U. Recent progress in clinical and basic pertussis research. Eur J Pediatr 2001;160:203-13.
19. Keitel W. Pertussis in adolescents and adults: time to reimmunize? Sem Resp Infect 1995;10(1):51-7.
20. Yaari E, Yafe-Zimerman Y, Schwartz SB, and others. Clinical manifestation of *Bordetella pertussis* infection in immunized children and young adults. Chest 1999;115(5):1254-8.
21. Herwaldt LA. Pertussis in adults. Arch Intern Med 1991;151:1510-2.
22. Strebel P, Stephen ML, Cochi K, and others. Pertussis in Missouri: evaluation of nasopharyngeal culture, direct fluorescent antibody testing, and clinical case definitions in the diagnosis of pertussis. Clin Infect Dis 1993;16:276-85.
23. Onorato IM, Wassilak GF. Laboratory diagnosis of pertussis: the state of the art. Pediatr Infect Dis 1987;6:145-51.
24. Farizo KM, Stephen L, Cochi ER, and others. Epidemiological features of pertussis in the United States, 1980-1989. Clin Infect Dis 1992;14:708-19.
25. Fine PEM, Clarkson JA. The recurrence of whooping cough: possible implications for assessment of vaccine efficacy. Lancet 1982;1:666-9.
26. Wright SWKME, Decker MD, and others. Pertussis infection in adults with persistent cough. JAMA 1995;273(13):1044-6.
27. Controlling pertussis in Texas (2004) Texas Department of Health. http://completecast.com/pertussis/ Accessed July 6, 2004.
28. Norman N. Pertussis trends in Texas. Texas Department of Health. http://www.tdh.state.tx.us/immunize/uparch/q104tren.htm Accessed July 6, 2004.
29. Centers for Disease Control and Prevention. Whooping cough, National Center for Health Statistics 2000.
30. Weiss AA, Hewlett EL. Virulence factors of *Bordetella pertussis*. Ann Rev Microbiol 1986;40:661-86.
31. Bassinet LPG, Maitre B, Housset B, and others. Role of adhesins and toxins in invasion of human epithelial cells by *Bordetella pertussi.* Inect Imm 2000;68:1934-41.
32. Kerr JR, Matthews RC. *Bordetella pertussis* infection: pathogenesis, diagnosis, management, and the role of protective immunity. Eur J Clin Microbiol Infect Dis 2000;19:77-88.
33. Orestein W. Pertussis in adults: epidemiology, signs, symptoms and implications in vaccination. Clin Infect Dis 1999;28(Suppl 2):S147-50.
34. Parton R. Review of the biology of *Bordetella pertussis*. Biologicals 1999;27:71-6.
35. Ladant D, Ullman A. *Bordetella pertussis* adenylate cyclase: a toxin with multiple talents. Trends in Microbiol 1999;7:172-7.
36. Keitel W. Cellular and acellular pertussis vaccines in adults. Clin Infect Dis 1999;28(S118-23).
37. DeVille JG, Cherry JD, Christenson PD, and others. Frequency of unrecognized *Bordetella pertussis* infections in adults. Clin Infect Dis 1995;21:639-42.
38. Villuendas MC, Lopez AI, Moles B, Revillo MJ. Infección por *Bordetella* spp: 19 años de diagnóstico por cultivo. Enferm Infecc Microbíol Clín 2004;22 (4):212-6.
39. Halperin SA, Bortolussi R, Wort AJ. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. J Clin Microbiol 1989;27:752-7.
40. Onorato IM, Wassilak SG. Laboratory diagnosis of pertussis:the state of the art. Pediatr Infect Dis J 1987;6:145-51.
41. Centers for Disease Control. Pertussis vaccination: use of acellualr pertussis vaccines among infants and young children. Recommendations of the Advisory Committee on Immunization Practices. MMWR 1997;46(RR-7):1-25.
42. Statistical Bulletin (1999). Immunization and Vaccine-Preventable Illness, United States, 1992-1997. p 16-7.
43. Senzilet LD, Halperin SA, Spika JS, and others. Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. Clin Infect Dis 2001;32:1691-7.
44. Robbins JB. Pertussis in adults: introduction. Clin Infect Dis 1999;28(Suppl 2):S91-3.
45. Halperin SA. Should all adolescents and adults be vaccinated against pertussis? Infect Med 2001;18(10):273-5.
46. Nenning ME, Shinefield HR, Edwards KM, and others. Prevalence and incidence of adult pertussis in an urban population. JAMA 1996;275:1672-4.
47. Jansen DG. Evaluation of pertussis in U.S. Marine Corps trainees. Clin Infect Dis 1997;25:1099-107.
48. Birkebaek NH, Kristiansen M, Seefeldt T, and others. *Bordetella pertussis* and chronic cough in adults. Clin Infect Dis 1999;29:1239-42.
49. De Serres GRS, Duval B, and others. Morbidity of pertussis in adolescents and adults. J Infect Dis 2000;182:167-79.
50. Dominguez AJV, Plans P, and others. The seroepidemiology of *B. pertussis* infection in Catalonia, Spain. Epidemiol Infect 2001;126:205-10.
51. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis 1990;161:480-6.
52. Mink CM, Cherry JD, Christenson P, and others. A search for *Bordetella pertussis* infection in university students. Clin Infect Dis 1992;14:464-71.
53. Gray GC, Callahan JD, Hawksworth AW, and others. Respiratory diseases among U.S. military personnel: countering emerging threats. Emerg Infect Dis 1999;5(3):379-87.
54. Brennan M, Strebel P, George H, and others. Evidence for transmission of pertussis in schools, Massachusetts, 1996: epidemiologic data supported by pulsed-field gel electrophoresis studies. The J of Infect Dis 1999;181:210-5.

Laboratory Managers' Views on Attrition and Retention of Laboratory Personnel

SUSAN BECK, KATHY DOIG

OBJECTIVE: This study was undertaken to provide information on the current shortage of clinical laboratory employees and to identify strategies for retaining laboratory employees.

DESIGN: A paper survey was distributed to 800 clinical laboratory managers.

SETTING: The survey was sent to laboratory managers at their work sites.

PATIENTS OR OTHER PARTICIPANTS: 190 usable surveys were returned for a response rate of 24%.

INTERVENTIONS: Surveys were mailed in March 2003.

MAIN OUTCOME MEASURES: The number of CLTs and CLSs considered fully staffed at the laboratory managers' institutions, the numbers of CLTs and CLSs who left the institutions in a five-year period, and the reasons employees left were tabulated. The managers' responses to questions on the factors that they considered most important in retaining laboratory employees were tabulated and categorized.

RESULTS: In this five-year period (1998-2002), 5% of employees left their jobs annually. Over 60% of laboratory employees who left did so in the first five years of practice. The top five reasons that employees left their jobs were: 1) new laboratory job, 2) moved/family obligations, 3) retirement, 4) left the field entirely, and 5) employee was fired. In the first year of practice, 15% of the employees who left were fired. Between one and five years of practice, 7.3% left because of the hours or shift, 6.7% left to pursue further education for a non-laboratory career, and 6.7% left the field entirely. In the group of employees who left between five and ten years, 13.5 % left the field entirely and 5.2% left for sales or clinical trials positions. Over 40% of the employees with more than ten years of experience who left did so because of retirement.

CONCLUSION: Most laboratory employees who left did so to take another laboratory position; however, reasons for leaving vary with years of experience. The number of laboratory employees leaving the profession exceeds the number of new graduates entering the profession making the retention of employees essential. Laboratory managers identified salary as the most important retention factor.

ABBREVIATIONS: ASCLS = American Society for Clinical Laboratory Science; ASCP = American Society for Clinical Pathology; BLS = Bureau of Labor Statistics; CLMA = Clinical Laboratory Managers Association; CLS = clinical laboratory science; CLSs = clinical laboratory scientists; CLTs = clinical laboratory technicians.

INDEX TERMS: clinical laboratory personnel; job satisfaction; personnel retention; personnel shortage; clinical laboratory staffing; workforce attrition.

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The clinical laboratory profession has been experiencing a personnel shortage for approximately 15 years.[1] Though the

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shortages subsided slightly in the mid-1990s, they returned dramatically at the end of the decade.[2] Renewed efforts in recruiting students and attention to retaining laboratory staff have lessened the shortage slightly as reported in the most recent surveys by the American Society for Clinical Pathology.[3,4] Such efforts will need to continue in order to maintain the laboratory workforce at current levels; however, it is unlikely that this level will meet the workforce needs of healthcare institutions in the future.

The Bureau of Labor Statistics (BLS) reports that about 297,000 individuals held positions as clinical laboratory scientists (CLSs) and clinical laboratory technicians (CLTs) in 2002.[5] Unfortunately, it is difficult to get an accurate count of clinical laboratory professionals because they are not licensed in most states. According to the BLS, a growth rate of 10% to 20% per year is expected through 2012 and this equates to a need for up to 59,000 new CLTs and CLSs over the next eight years, or nearly 7,500/year. Although the BLS estimate takes into account average rates of attrition including retirements, the retirement rate is expected to climb as the baby boom generation retires. National professional organizations such as the American Society for Clinical Laboratory Science (ASCLS) estimate the age of their average member to be in the late 40s (unpublished data, 2004). Therefore, the BLS estimate of 7,500 new laboratory professionals needed each year is probably too low.

The majority of new laboratory professionals will be graduates of formal CLT or CLS educational programs. In 2003, the number of graduates of educational programs accredited by the National Accrediting Agency for Clinical Laboratory Science (NAACLS) was approximately 4,000.[6] In the past few years, national organizations and educational programs have increased recruitment efforts and many programs have seen an increase in the number and quality of students.[7] Even if all programs were fully enrolled and all the students in the programs graduated, it is unlikely that the number of graduates would meet the projected need for 7,500 new laboratory professionals. It is clear that the current personnel shortage must also be addressed by improving the retention of laboratory employees.

Previous studies of the clinical laboratory workforce have discussed the connection between employee retention and personnel shortages. In a survey of laboratory managers, Gardner and Estry determined that turnover, rather than reduced entrants, was the major factor contributing to the shortage at the end of the 1980s.[8] They also projected that a low entry rate to the profession would exacerbate shortages by the end of the 1990s.[9] Other surveys of laboratory managers have yielded recommendations for improving employee satisfaction and retention including increasing salaries, improving recognition, improving opportunities for career advancement, reducing job stress, improving work hours, and allowing staff more control over their work.[10,11]

Surveys of laboratory practitioners have been consistent in identifying salary as a major issue in employee satisfaction and retention.[12-17] Poor benefits, job stress, limited advancement opportunities, and lack of recognition are also often mentioned as reasons people are dissatisfied and likely to leave the clinical laboratory profession. In a 2003 survey of laboratory practitioners, salary topped the list of factors considered important for employee retention.[18] The lack of parity with health professionals who have similar education and experience was the chief source of dissatisfaction with salary. Although not as strongly felt, the second most commonly mentioned source of dissatisfaction was lack of recognition from physicians and nurses. Practitioners also cited the short staffing that leads to long work hours as contributing to employee attrition.

This study was undertaken to provide information on the current shortage of clinical laboratory employees and to identify strategies for retaining laboratory employees. A national survey of laboratory employees who left their jobs would be the most informative in understanding the magnitude of the problem and the reasons for attrition. Because that population cannot be easily reached, a survey of laboratory managers was used to obtain their perspective on why their employees left. Specifically, this study addressed the following questions:

1. Why do laboratory practitioners leave (from their managers' perspectives)?
2. How many are leaving and when are they leaving?
3. Are reasons for leaving different based on the number of years practitioners have worked, the institution size, or the level of practice (CLT or CLS)?
4. What strategies do laboratory managers think are most effective in retaining employees?

MATERIALS AND METHODS

The researchers prepared a survey, list of definitions, and cover letter for clinical laboratory managers with questions related to the attrition and retention of CLT and CLS practitioners. Laboratory managers were asked to provide the number of CLTs and CLSs that is considered fully staffed at their institu-

tion. For each employee who left in the five-year period (1998-2002), managers were asked to provide the practitioner's level of practice (CLT or CLS), years of employment, and reason for leaving. Laboratory managers were also asked to identify the factors that they felt were most important for employee retention and describe the policies or practices that they found most successful in retaining employees. The survey also included demographic questions on geographic location, type of work facility, size of institution, primary job function, licensure, gender, ethnicity, highest degree, and years of experience. To group geographic locations, the American Society for Clinical Laboratory Science (ASCLS) regions were used.

The survey, cover letter, and definitions were reviewed by an advisory board comprised of laboratory managers, practitioners, and educators. The survey was tested in a pilot study using a convenience sample of laboratory managers known to the researchers. The results of the pilot study were reviewed and the survey was revised based on these suggestions. The survey and cover letters were approved by the University Committee on Research Involving Human Subjects of Michigan State University, E Lansing, MI.

The managers selected for the study were identified from the mailing list of the Clinical Laboratory Managers As-

Table 1. Supervisors' perceptions of the reasons employees left laboratory positions between January 1, 1998 and December 31, 2002 grouped by years of experience

	Percent of employees who left				
	All employees (n = 1039)	**Left ≤1 year (n = 223)**	**Left >1 and ≤5 years (n = 356)**	**Left >5 and ≤10 years (n = 155)**	**Left >10 years (n = 174)**
New laboratory job (technical or administrative)	**31.7***	**32.3***	**34.6***	**35.5***	**19.0***
Family obligations/moved	**18.1**	**19.7**	**21.1**	**19.4**	**9.2**
Retirement	**9.2**	0.4	0.8	4.5	**42.4**
Left laboratory field entirely	**8.7**	**5.8**	**6.7**	**13.5**	**11.5**
Fired	**6.4**	**15.2**	5.1	3.2	2.3
Hours (shift, wanted FT or PT)	6.1	**6.7**	**7.3**	3.9	3.4
Further education for a non-laboratory career	5.0	4.0	**6.7**	**4.5**	2.3
Sales or clinical trials	4.0	1.7	4.8	**5.2**	3.4
No reason given	1.9	4.6	1.7	2.6	0.0
Other (stress, commute, wanted patient contact, called to military service)	2.3	2.2	2.6	3.2	0.6
Health, personal reasons, deceased	2.8	3.2	2.5	1.9	**4.6**
Sought better salary	1.8	1.4	3.1	1.9	0.6
Further education in laboratory field	1.2	2.3	1.7	0.0	0.0
Technical position in a research laboratory	0.8	0.5	1.3	0.7	0.7

* The top five reasons in each group are in bold print.

sociation (CLMA). They were selected by choosing every sixth name from the zip code sorted list. To maximize the likelihood that the survey recipient would be a laboratory manager, individuals whose place of employment or job title suggested they were not managing a laboratory were deleted. Eight hundred managers were selected for the final mailing. The surveys were sent in March 2003, with cover letters and postage-paid return envelopes. Two weeks after sending the manager packets, a follow-up reminder postcard was sent to all managers.

Data analysis

SPSS 11.5 was used to analyze the data collected in this study. The response rate was calculated by dividing the number of usable surveys returned by the total number mailed. Descriptive statistics were used to tabulate responses and calculate means. The number and percent of employees who left laboratory positions for each of 14 different reasons were tabulated (Table 1). Employees were further grouped by years of experience, size of institution, and certification level. In each of these groups, the employees' reasons for leaving were tabulated and the top five reasons were identified. Because of the small number of employees in some categories, Chi square analysis was not used to analyze differences among these groups. Participants' written responses to the question, "What factors do you think are most important in retaining qualified clinical laboratory practitioners in your laboratory?" were tabulated and grouped into major categories by the researchers.

RESULTS

Response

A total of 190 usable surveys were returned which represents a 24% response rate.

Demographic information on respondents

The laboratory managers came from all geographic regions of the country. The highest percentage of responding managers (16.5 %) came from the ASCLS Region IV (MI, IN, OH, KY) and the lowest percentage of responding managers (2.1%) came from ASCLS Region VIII (CO, ID, MT, UT, WY). In the other ASCLS regions, the percentage of responding managers ranged from 8.0 % to 15.4%.

Most of the laboratory managers worked in hospitals or medical centers (77.9%). The next largest percentage worked in physician office or group practice laboratories (10.5%) or reference laboratories (5.3%). A small percentage of the respondents indicated that they worked in academic health centers (1.6%) or HMOs (0.5%).

To assess institution size, laboratory managers were asked for the annual volume of tests performed in their clinical laboratories. Approximately 13% of the laboratory managers worked in institutions with annual test volumes of less than 100,000. Most respondents (44.1%) were from institutions with annual test volumes between 100,001 and 500,000. One fourth of the laboratory managers worked in institutions with test volumes between 500,001 and 1,000,000 per year and 18.1% worked in institutions with test volumes greater than 1,000,000 per year.

The survey respondents identified their job functions as Laboratory Manager/Administrator/Director (86.3%), Laboratory Supervisor (10.5%), or CLS (2.1%). The majority of the laboratory managers were not licensed (76.3%). The managers were primarily female (71.1%) and Caucasian (93.7%). The ethnic group selected by the second highest percent of respondents was Asian (2.6%). None of the laboratory

Table 2. Laboratory managers' academic degrees grouped by years of experience

	Laboratory managers with ≤25 years of experience		Laboratory managers with >25 Years of experience		Total	
Degree	#	%	#	%	#	%
Associate	0.0	0.0	8	7.9	8	4.2
Baccalaureate	50	57.5	64	62.7	114	60.3
Master's Degree	35	40.2	29	28.4	64	33.9
Doctorate	2	2.3	1	1.0	3	1.6
Totals	87	100	102	100	189	100

managers were African American and a small number were Hispanic (0.5%) and Native American (1.6%).

The laboratory managers' years of experience ranged from 8 to 45 years with a mean of 26.6 years (SD = 7.06). Laboratory managers had worked for a mean of 9.5 years in their current jobs. Approximately sixty percent (60.3%) of managers listed the baccalaureate degree as their highest degree. One third of the laboratory managers had a Master's degree and 1.6% had a doctoral degree. Some respondents (4.2%) listed the Associate Degree as their highest degree. Laboratory managers were separated into two groups; those with 25 years of experience or less and those with more than 25 years of experience. The number and percent of managers in each group and their degrees is shown in Table 2.

Attrition

Laboratory managers were asked to list the number of CLTs and CLSs that is considered 'fully staffed' in their institutions. One hundred eighty five (185) laboratory managers responded to this question and listed 1688 CLTs and 2499 CLSs for a total of 4187 employees. The average number of employees (CLTs and CLSs) in these 185 institutions was 22.6. The laboratory managers identified 1046 employees who left their institution in the five-year period between January 1, 1998 and December 31, 2002. Two hundred fifty eight (258) CLTs, 733 CLSs, and 55 employees who were not identified as CLTs or CLSs left these institutions. The employees who left were employed for a mean of 6.7 (SD = 7.8) years before they left. Approximately 25% of the employees who left, did so in the first year of practice. Over sixty percent (63.9%) of the employees who left, did so in five years or less.

Table 3. Supervisors' perceptions of the reasons employee left their laboratory positions between January 1, 1998 and December 31, 2002 grouped by institution size and certification

	Percent of employees who left			
	Annual test volumes		Certification of employees	
	< = 500,000 (n = 391)	>500,000 (n = 617)	CLT (n = 256)	CLS (n = 733)
New laboratory job (technical or administration)	**34.5***	**29.4***	**28.6***	**32.3***
Family obligations/moved	**14.8**	**19.9**	**18.0**	**18.2**
Retirement	7.4	**10.8**	5.1	**10.8**
Left laboratory field entirely	**9.0**	**9.0**	7.4	**8.8**
Fired	**6.9**	6.2	**10.2**	5.2
Hours (shift, wanted FT or PT)	5.4	**6.7**	**9.8**	4.6
Further education for a non-laboratory career	4.9	5.1	4.3	**5.3**
Sales or clinical trials	3.1	4.7	1.6	4.9
Other (stress, commute, wanted patient contact, called to military service)	4.1	1.2	2.4	2.3
No reason given	1.8	1.3	3.4	1.6
Health, personal reasons, deceased	5.3	1.3	3.4	2.4
Sought better salary	0.8	2.6	3.4	1.4
Further education in laboratory field	1.0	1.1	2.0	1.1
Technical position in a research laboratory	1.0	0.7	0.4	1.1

* The top five reasons in each group are in bold print

The laboratory managers' perceptions of the reasons that employees left in the five-year period between January 1, 1998 and December 31, 2002 are listed in Table 1. Laboratory managers listed reasons for 1039 of the 1046 employees who left. The highest percentage of employees (31.7%) left to take a new technical or administrative position in the laboratory. The next highest percentage of employees (18.1%) left because of family obligations or because they moved. Retirement was listed as the reason for 9.2% of the employee attrition. Approximately nine percent (8.7%) left the laboratory field entirely and 6.4% were fired.

Laboratory managers were asked to indicate how long an employee had worked for them before leaving. Managers provided this information for 908 of the employees who left. The reasons for leaving were tabulated for four groups of employees; 1) those who had worked one year or less, 2) those working between one and five years, 3) those working between five and ten years, and 4) those who had worked more than ten years (see Table 1). In the first three groups, most employees left for a new laboratory position or because of family obligations/relocation. Approximately 15% of employees who left in the first year of practice, did so because they were fired. The reasons employees who had worked between one and five years left included the hours or shift (7.3%), the pursuit of education for a non-laboratory career (6.7%), and a decision to leave the field entirely (6.7%). In the group of employees who left between five and ten years, 13.5% left the field entirely and 5.2% left for sales or clinical trials positions. In the fourth group, those who worked for more than ten years, the highest percentage of employees left for retirement (42.4%).

The reasons employees left were also tabulated for employees from institutions with volumes of 500,000 tests/year or less and those from institutions with volumes greater than 500,000 tests/year (see Table 3). A higher percentage of employees from smaller institutions than employees from larger institutions left for health or personal reasons (5.3% vs. 1.3%). Reasons for leaving were also tabulated for employees certified as CLTs and those certified as CLSs (Table 3). A higher percentage of CLSs than CLTs were retiring (10.8% vs. 5.1%) and leaving for sales/clinical trials positions (4.9% vs. 1.6%). More CLTs than CLSs were fired (10.2% vs. 5.2%).

Given a list, the managers were asked to identify policies or practices that were most successful in retaining employees in their laboratories. The top ten policies or practices and the percentage of managers selecting each policy were:

1. Raised salaries (36.5%)
2. Adjusted employee hours to fit family obligations (18.9%)
3. Gave employees more responsibility for day to day decisions (15.3%)
4. Spent more time praising employees for good performance (8.4%)
5. Involved laboratory staff in new projects, e.g., method comparison (7.9%)
6. Actively recruited new staff to fill vacancies (7.9%)
7. Resolved personnel conflicts (6.8%)
8. Improved the physical environment of the laboratory (5.8%)
9. Increased overtime pay or shift differentials (5.3%)
10. Developed employee appreciation programs (4.2%).

Managers also responded to the question, "What factors do you think are most important in retaining qualified clinical laboratory practitioners in your lab?" This open-ended question allowed managers to express their opinions and add retention factors that may not have been addressed in the list provided on the survey. The ten factors that were mentioned most often by the managers are listed below with the percentage of managers listing that factor:

1. Salaries and benefits that are competitive with other institutions and other professions with comparable education and responsibility (67%)
2. Flexible scheduling, no weekends, no shift work (35%)
3. Positive feedback, praise, recognition for work (22%)
4. Involvement in decision making, individual responsibility, and control (22%)
5. Good management team (20%)
6. Good co-workers, good team work (18%)
7. Recognition and respect from nursing, administration, pathologists, and the public (16%)
8. Work that is challenging, satisfying, interesting, varied work (14%)
9. Good work environment (14%)
10. Adequate staffing, good workload, reduced stress (14%).

DISCUSSION

The response rate for this survey was 24%, which is comparable to similar surveys of laboratory managers.[9,19] The laboratory managers came from all geographic locations and from institutions of all sizes. Most were from hospitals or medical centers and 98% listed their job function as supervisor, director, or administrator. The survey method, therefore, appeared to be successful in reaching the target population

and providing results that are representative of views of the laboratory managers across the country.

The laboratory managers in this study were predominately women (71%) and most had a baccalaureate degree (60.3%). Previous studies documented a lower percentage of women in laboratory management. A 1980 ASMT National Compensation Survey reported that 32% of the managers or supervisors were women and a 1989 ASMT national survey found that 55% of the laboratory managers were women.[9,20] The percentage of women in management has increased in the past 25 years and is now similar to the percentage of women in the overall population of laboratory employees.[21] Over 40% of the laboratory managers with 25 years of experience or less had obtained an advanced degree compared to 29.4% of the laboratory managers with more than 25 years of experience. A master's or a doctoral degree was not typically needed for laboratory management positions in the past; however, younger managers may have found the advanced degree helpful in preparing for management positions and competing for job openings.

Laboratory managers report that approximately 25% of the employees who left did so in the first year of practice and over 60% of those who left did so in five years or less. A higher percentage of employees leaving early is not surprising because in the first few years of practice, employees may be learning whether or not the clinical laboratory environment is a good match for them. It is also a time when employees may need mentoring and close supervision to ensure that new employees are given as much assistance and encouragement as possible. The problem of losing employees early in their careers is not unique to the clinical laboratory profession. A national report on attrition in teaching states that almost one-third of teachers in the United States leave the field within the first three years of practice and half of the teachers leave before their fifth year.[22]

This study attempted to determine how many laboratory employees are leaving the profession. Laboratory managers in these 185 institutions reported that a total of 4187 CLT and CLS employees would be considered fully staffed. They also reported that a total of 1046 employees left their institutions in a five-year period. If 1046 employees left in a five-year period, an average of 209 employees left each year. This represents 5% of the total population (4187 employees) reported by these laboratory employers. The Bureau of Labor Statistics reported that there were 297,000 laboratory employees in 2002.[5] If 5% of those 297,000 laboratory employees left, this would create 14,850 vacancies each year. According to the additional information collected in this survey, approximately 32% of the employees left for new laboratory positions. So, although 14,850 laboratory positions may be vacated each year, not all of those employees left the laboratory profession. If the number of laboratory employees who are leaving is adjusted for the 32% of employees who left for new laboratory positions, the estimated number of people leaving the clinical laboratory annually is 10,098.

Any estimate of the number of laboratory employees leaving the field will be flawed because reliable data on the current laboratory workforce are not available. Nevertheless, both of the estimates in this study and the BLS estimate of the number of new professionals needed are higher than the number of students graduating from CLT and CLS programs each year. The results of this study underscore the critical need for retention of laboratory employees.

The top five reasons that employees left their jobs according to the laboratory managers were: 1) left for a new laboratory job, 2) moved or left due to family obligations, 3) retirement, 4) left the field entirely, and 5) the employee was fired. A review of the reasons for leaving for employees with varying years of experience provided additional descriptive information that may help managers address retention issues. For laboratory employees who had worked for ten years or less, more than half of the employees left for the first two reasons (new laboratory job or moved). The first reason, leaving for a new laboratory job, is a positive sign. This may indicate that employees are finding opportunities that better fit their interests or they have advanced in their careers. Although an employee created a vacant position for one manager, the individual was retained in the laboratory profession. The second reason for leaving, moving/family obligations, also leaves a vacancy and if the individual moves to a new area and seeks another laboratory position, the individual may not be lost from the profession. Laboratory managers may be able to help retain employees in the profession if they provide networking information for employees who move.

Approximately 15% of the employees who left in the first year of practice did so because they were fired. The percentage of employees who were fired was higher in this group than in groups with more than one year of experience. This would seem to indicate that laboratory managers are dealing with problems early in their employees' careers and removing employees who can't handle the work of the clinical laboratory. This sets a standard of excellence for all employees and

contributes to all employees' pride in their work. There are other possible explanations for the high percentage of employees who were fired in the first year of practice including insufficient orientation or training and unrealistic expectations of the work.

The highest number (356) of employees in this study who left, did so between one and five years. In this group, 7.3% left because of the hours, 6.7% left to pursue further education for a non-laboratory career and 6.7% left the field entirely. Those dissatisfied with their shift or hours may have taken an undesirable shift in an entry-level position with the hope that they would soon move to better shift. If that did not happen in the first five years of practice, the employee may have decided to seek other career opportunities. Those leaving for further education or leaving the field entirely may not have been challenged in their current positions.

The higher loss of employees in the first five years may also be a reflection of differences between what the laboratory environment provides and what younger employees find satisfying. Further research is needed to assess the degree to which generational value differences influence retention factors. Because this is a crucial time for employee retention, laboratory managers should pay close attention to these employees' level of satisfaction with their hours and their job responsibilities. This is also an important time to provide a mentor for laboratory employees. The opportunity to discuss career options, frustrations, and future plans with an experienced laboratory professional may help these younger employees deal with difficulties and make a commitment to the laboratory profession.

The results of this study indicate that fewer employees are lost after five years, but because these employees have additional years of experience and clinical expertise, their loss may be more significant for an institution than the loss of younger employees. In the group of employees who left between five and ten years, 13.5% left the field entirely. Although managers reported that only approximately 2% of employees in this group left because they were seeking better salaries, the desire for a higher salary may have been an underlying factor in the decision to leave the field.

The highest percentage of employees who left because of retirement was in the fourth group of employees, those who left after working for more than ten years. The high percent of people leaving for retirement in this group (42.5%) is consistent with the descriptions of the aging clinical laboratory workforce and there is little managers can do to prevent this type of attrition. The lowest percentage of employees in this group left for new laboratory positions indicating that after ten years, employees may be committed to their jobs until retirement.

A higher percent of CLS employees left for retirement than CLT employees, possibly because there were not as many graduates of CLT programs 30 years ago. More CLSs also left for positions in sales and marketing possibly because those positions require a baccalaureate degree. A higher percent of CLT employees were fired than CLS employees. Additional information would be needed to understand this difference; however, one possible explanation is that some employees classified as CLTs by the managers in this survey had job titles of "clinical laboratory technician" but did not have formal education or hold national certification as a CLT.

The managers in this study were very aware of the importance of retaining laboratory employees. In their responses to questions on the factors and practices that they thought contributed to employee retention, four issues were mentioned most often. They were salary, scheduling, staffing, and supervision/management. In the classic work of Herzberg on employee motivation, factors contributing to employee satisfaction and motivation were distinguished.[23] Hygiene factors, such as supervision, work conditions, interpersonal relationships, and salary are necessary for satisfaction but not sufficient for motivation. Whether guided by this research or not, laboratory managers appear to appreciate that satisfaction with hygiene factors is essential to retention. The factors that managers listed most often as important in employee retention were hygiene factors such as raising salaries, flexible scheduling, adequate staffing, and good supervision.

Herzberg's findings suggest that satisfaction with hygiene factors alone will not be sufficient to address employee retention. Commitment to the clinical laboratory profession would be expected to depend on motivational factors like achievement, recognition, responsibility, and advancement opportunities. Managers' responses demonstrate that they also value motivational factors. For example, one manager listed the following factors as important for retention; "opportunity for continuing education and promotion, some degree of self-governance, a climate of trust and respect and open frequent communication, appreciation, team process improvement and a culture where fun is OK and there is no fear."

Yet Herzberg's work would predict that efforts in these motivational areas will be effective only with employees who are

already satisfied with the hygiene factors. Unless laboratory managers address the hygiene factors, their efforts to motivate and instill the professional commitment necessary for retention will be undermined. This represents a significant challenge to laboratory managers and healthcare institutions because it will call for additional resources to raise salaries and hire enough laboratory practitioners to improve scheduling issues. The salary challenge in particular may be greater than some managers appreciate because merely raising salaries higher than current levels may not be adequate. Laboratory employees are acutely aware that their salaries have fallen below that of other healthcare professionals and satisfaction with salaries will mean compensation that is equivalent to healthcare professionals with similar education and experience.[18]

CONCLUSIONS

A description of the reason why laboratory employees left over a five-year period showed that most employees who left their jobs, did so to take a new laboratory job. This is an encouraging finding indicating that these employees are staying in the profession and making a further commitment to their laboratory careers. The second highest percentage of employees who leave, are doing so because of a move, usually for family reasons. There is little laboratory managers can do about this group, other than help them make connections with laboratory managers in their new location.

Given that there are some things that laboratory managers can do nothing about such as employees moving, it is important to look at the other reasons employees leave to see if managers can influence those employees. Most laboratory employees who leave are doing so in the first five years of practice and, in addition to leaving for new laboratory jobs and leaving because of moving, employees in this group left because of the shift or hours and to pursue further education. Laboratory managers should work to monitor employees' concerns about hours and provide a work environment that provides challenges and opportunities for continued learning. Although fewer employees leave after five years, they take a great deal of expertise with them when they leave and many of these employees are leaving the field entirely. Many institutions have salary bonuses to attract new employees; however, a plan for rewarding experienced employees is just as important to prevent a 'brain drain' in the clinical laboratory.

This study represents a first step in understanding why employees leave the clinical laboratory in the first decade of the 21st century. Additional studies are needed to better understand this complex issue and assess differences among groups of employees. Studies that collect data directly from employees who left laboratory positions are needed as well as studies that collect parametric data and provide statistical comparisons among groups. Studies of employee attrition across multiple disciplines would also be helpful in identifying common problems and shared solutions.

In every question on this survey on strategies for retaining employees, laboratory managers listed salaries as the most important retention factor. Hospital administrators have responded to shortages in nursing and radiologic technology with higher salaries, but there has not been a similar response to the shortage of laboratory personnel. This may be because the laboratory is behind the scenes and the impact on patient care is not as evident as that of nursing or radiologic technology. Also, laboratory employees often work extra hours to make sure that laboratory results are available and accurate and this mitigates the effect of the shortage on the institution. The shortage of laboratory personnel does, however, have an effect on the institutions' finances when the costs of recruiting and new employee orientation are considered and this should be documented for hospital administrators.

In a study of one medical center, turnover costs represented about 5% of the annual operating costs which was equivalent to giving every nurse on staff a 33% retention supplement each year.[24] Laboratory managers need to make the case to administrators in their institutions that until the salary issue is addressed, laboratory employees will continue to leave and the numbers cannot be entirely replaced by new graduates. However, the work of improving salaries is not the sole responsibility of the laboratory managers. Laboratory employees must help administrators understand the value of the clinical laboratory by presenting a professional image at all times and by contributing to institution-wide committees and projects. In addition, evidence that timely, accurate laboratory tests improve patient care and shorten length of stay must be collected to support efforts to raise salaries.

This study provided an estimate of the number of people who are leaving the profession each year. Although this estimate is limited by the lack of good data on the current number of laboratory professionals, it far exceeds the number of students graduating from CLT and CLS programs each year. Continued recruiting efforts are needed, and in some areas, more educational programs may be indicated; however, this problem will not be solved simply by educating more CLT and CLS students. Workplace issues directly affect recruitment and savvy students will not choose educational programs that lead to undesirable careers.

If talented young people are not attracted to the laboratory profession and competent practitioners are not retained in the laboratory workforce, the quality and availability of laboratory services will decline. The personnel shortage has led to 'stop gap' measures such as sign on bonuses, hiring non-certified personnel, and asking more of current employees. These short-term approaches may mean that the laboratory functions for one more day, but they do not help retain laboratory practitioners. Creating an environment in which laboratory practitioners are compensated for their education and experience and have opportunities for continued professional growth is essential for the future of the laboratory profession and for the health of the public.

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REFERENCES

1. Castleberry BM, Wargelin LL. 1998 wage and vacancy survey of medical laboratories. Lab Med 1999;30:174-8.
2. Ward-Cook K, Tannar S. 2000 wage and vacancy survey of medical laboratories. Lab Med 2001;32:124-38.
3. Ward-Cook K, Chapman S, Tannar S. 2002 wage and vacancy survey of medical laboratories part II: Modest easement of staffing shortage. Lab Med 2003;34:702-7.
4. Ward-Cook K. Preliminary Results of the ASCP 2003 Wage and Vacancy Survey. http://www.ascp.org/bor/center/wage_vac2003prel.asp. Accessed August 1, 2005.
5. *Occupational Outlook Handbook, 2004-05 Edition*, Clinical Laboratory Technologists and Technicians, Bureau of Labor Statistics, U.S. Department of Labor. http://www.bls.gov/oco/ocos096.htm. Accessed August 1, 2005.
6. National Accrediting Agency for Clinical Laboratory Sciences. Accreditation Update. Clinical Laboratory Educators' Conference. Milwaukee WI. March 2004.
7. Ward-Cook, Daniels K, Gueriguieva M. ASCP Board of Registry's 2001 Annual Survey of Medical Laboratory Science Programs. Lab Med 2002:33:831-6.
8. Gardner PD, Estry DW. Changing job responsibilities in clinical laboratory science: a report on the 1989 ASMT National Personnel Survey. Clin Lab Sci 1990;3:382-8.
9. Estry DW, Gardner PD. Personnel status trends in clinical laboratory science: a report on the 1989 ASMT National Personnel Survey. Clin Lab Sci 1990;3:258-67.
10. Karni KR, Feickert JD. Occupational reinforcers for medical technologists in clinical laboratories – 1986-1987. Clin Lab Sci 1989;2:355-61.
11. Estry DW, Gardner PD, Nixon D. Factors affecting laboratory turnover and recruitment: The 1989 ASMT National Personnel Survey with emphasis on salary comparisons to 1991 data. Clin Lab Sci 1992;5:96-103.
12. Guiles JH, Lunz ME. A comparison of medical technologist salaries with other job categories and professions. Lab Med 1995:26(1):20-2.
13. Hofherr LK, Francis DP, Peddecord KM, Krolak JM. A census survey and profile of clinical laboratory scientists, University of Minnesota. Lab Med 2003;34:29-34.
14. Harmening DM, Castleberry BM, Lunz ME. Technologists report overall job satisfaction: 10-year retrospective study examines career patterns. Lab Med 1994;25:773-5.
15. Lunz ME, Morris MW, Castleberry BM. Medical technologist career commitment and satisfaction with job benefits. Clin Lab Man Rev 1996;(N/D):613-8.
16. Trotto NE. Job satisfaction in the field: women speak out. Med Lab Observ 1992; June:22-8.
17. Ringel MJ. Parenthood, harassment, and other workplace distractions. Med Lab Observ 1992; June:29-33.
18. Doig K, Beck S. Factors contributing to the retention of clinical laboratory personnel. Clin Lab Sci 2005;18(1):16-27.
19. Doig K, Beck SJ, Kolenc K. CLT and CLS job responsibilities: current distinctions and updates. Clin Lab Sci 2001;14(3):173-82.
20. ASMT Education & Research Division. The American Society for Medical Technology 1979 national compensation survey. Am J Med Tech 1980,46:191-9.
21. Ward-Cook K, Tatum D, Jones G. Medical technologist core job tasks still reign. Lab Medicine 2000;31:376.
22. The National Commission on Teaching and American's Future. Unraveling the "Teacher Shortage" Problem: Teacher Retention is the Key. Washington, DC August 20-22, 2002. http://www.nctaf.org/article1?c=4&sc=17&.ssc=o&a=229. Accessed August 1, 2005.
23. Herzberg F, Mausner B, Snyderman BB. The motivation to work. New York: Wiley. 1959.
24. Waldman J, Kelly F, Arara S, Smith H. The shocking cost of turnover in healthcare. Healthcare Manage Rev 2004:29:2-7.

A Qualitative Assessment of Systematic Instructional Design Training by CLS Faculty Members

VICKI FREEMAN, CAROL LARSON, J DAVID HOLCOMB

OBJECTIVE: To determine the perceived value clinical laboratory science (CLS) faculty members gave to their participation in workshops on the use of a modified systematic instruction design (SID) model to develop curriculum and on-line courses.

DESIGN: A survey assessing the perceived value of SID training was sent to 27 CLS faculty members. The survey asked the respondents to assess the value of the training that they received in developing their skills in Web-based, distance learning course development and teaching, and expanding their skills in traditional course development and teaching. The eight components of SID were listed and the respondents rated each component as to its value to them on a 5-point Likert scale of 5 = very valuable to 1 = not very valuable. In addition to rating the value of each SID component, the respondents were asked if they would like more training in any of the eight components.

RESULTS: A majority of the18 respondents (67%) reported that the training in SID was valuable to them. A strong majority of the respondents indicated that their training in goal and instructional analyses (96%), media selection (94%), and aligning objectives, assessments, and instructional strategies (94%) were valuable to their distance education programs and their traditional teaching skills.

CONCLUSION: Faculty members who actively participated in SID training valued their new skills in developing distance education courses as well as improving their traditional teaching activities. Research is needed on the effect these new teaching skills have on student learning.

The peer-reviewed Research and Reports Section seeks to publish reports of original research related to the clinical laboratory or one or more subspecialties, as well as information on important clinical laboratory-related topics such as technological, clinical, and experimental advances and innovations. Literature reviews are also included. Direct all inquiries to David G Fowler PhD CLS(NCA), Clin Lab Sci Research and Reports Editor, Dept of Clinical Laboratory Sciences, University of Mississippi Medical Center, 2500 North State St, Jackson MS 39216. (601) 984-6309, (601) 815-1717 (fax). dfowler@shrp.umsmed.edu

ABBREVIATIONS: CETs = content expert teams; CLS = clinical laboratory science; SID = systematic instructional design; WBE = Web-based education; WebCLS = Web-based education in clinical laboratory science.

INDEX TERMS: clinical laboratory sciences; faculty development; systematic instructional design; Web education.

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Web-based education (WBE) is a form of distributed learning in which the WWW, Internet, and intranets are used as the vehicle for delivering the training to learners anywhere and at any time. WBE is transforming the delivery of education at all levels, from kindergarten through post-graduate education. It allows educators to bring learning to students instead of bringing students to learning. By defying the constraints of time and distance, WBE education makes it possible for more individuals than ever to access knowledge, to learn in new and different ways, and to embrace lifelong learning. However, when reviewing the current proliferation of Web-based courses, one finds that they either mimic traditional correspondence mail models or simply make traditional lecture based materials available on-line.
According to Mishara, most Web-courses are "nothing more

than classroom lecture materials posted on to the Web".[1] Carr-Chellman and Duchastel state that "many on-line courses lack basic design considerations and that the Web is simply being used as a medium for the delivery of instruction created within another framework".[2] As a result, while educational opportunities are increasing for students, the quality of the educational experience is not necessarily increasing. This medium is capable of supporting a wide range of multimedia technologies, making it an ideal environment for learning. Web-based instruction can make use of hyperlinking, synchronous and asynchronous conferencing and interactions, real-time audio and video, and even 3-D virtual reality that are generally not possible with traditional computer or classroom delivery alone. Other advantages of WBE include: accessibility (platform-independent and to world-wide audience); relatively low development and distribution costs; ability to link to other programs and resources; interactivity possibilities; ability to restrict use, if desired; and ability to provide just-in-time learning 'on-location'.[3] The availability of e-mail, on-line discussion boards and chats increases the ease of interaction between classroom participants.[4] However, the instructional materials to be used in this type of medium must be designed with these characteristics in mind in order to construct quality course modules.

Delivery of education via the Web can enable educators to center learning on the student instead of the classroom and to focus on the strengths and needs of the individual learner. However, according to the Web-based Education Commission (WBEC), "The power of the Internet to transform the educational experience is awe-inspiring, but it is also fraught with risk".[5] The Commission found that teachers were the key to the effective use of Web-based tools and applications. However, almost two-thirds of all teachers felt they were either not at all prepared or only somewhat prepared to use technology in their teaching.[6] In 1999, 70% of educators polled regarding technology in instruction put professional development at the top of their list of technology challenges.[4] Respondents reported that both initial training for those just beginning to use technology and on-going training to support the growth of innovators were needed. Five consecutive years of surveys in higher education showed the same thing: institutions ranked their greatest technological challenge as "assisting faculty to integrate information technology into instruction".[7] WBEC's Report included the need to provide continuous and relevant training and support for educators and to develop high quality online educational content that meets the highest standards of educational excellence.[4]
During traditional classes, a good educator can often make up for poorly designed instructional materials by facilitating or adding unplanned interactions that were not designed as part of the original teaching materials. When the teacher is removed from the immediate classroom, it becomes essential that key online interactions are designed and planned. According to Hirumi, in WBE, "opportunities to interact and adapt instruction based on spontaneous verbal and nonverbal cues are relatively limited. Furthermore, the use of interactive technologies does not ensure that interactions will take place. Key interactions must be planned and sequenced if they are to occur consistently as an integral part of WBE".[8]

Ely stated that "there is growing evidence that the use of instructional design procedures and processes leads to improved learning without regard to the hardware and software that is used".[9] He believes that the design of instruction is "a more powerful influence on learning than the system that delivers the instruction". What is instructional design? According to Wilson, "instructional design involves the preparation, design, and production of learning materials".[10] The instructional design process includes establishing learning goals and objectives, the methods to assess the learning outcomes and the content, learning interactions, and student activities to help the student progress in attaining the goals and objectives. The purposes of SID are to improve learning and instruction through a variety of means including problem-solving and feedback, management of the design and development, improving evaluation processes and testing and/or building learning by instructional theory.[11]

In 1999, the Department of Clinical Laboratory Sciences at The University of Texas Medical Branch received a grant from the Department of Education to pursue the development of a Web-based curriculum. The project, titled Web-based Education in Clinical Laboratory Sciences (WebCLS), was carried out over a three-year period. The goal of the project was to develop, implement, and evaluate an interactive Web-based curriculum model for baccalaureate-level clinical laboratory science (CLS) education. This model was to include Web-based course materials, on-line interactive course laboratories using video and animation, interactive discussion and chat sessions, on-line testing and evaluation, comprehensive on-line review materials, and a pilot demonstration of a virtual practice laboratory. Ten collaborating institutional partners supported the development and dissemination of this interactive model for laboratory oriented Web-based educational instruction including CLS and CLT faculty members from four universities and three community colleges, faculty and graduate students from an instructional technology graduate

program, and representatives from the Southern Regional Education Board (SREB), and from the National Laboratory Training Network (NLTN).

Faculty members from collaborating institutions participated in a series of training sessions designed to guide them in the design and development of interactive, student-centered courses. With this training, faculty members were empowered to develop and maintain their course Web sites.

This paper presents information on 1) how the SID process was used to prepare CLS faculty members to develop Web-based, distance education; and 2) the perceived value CLS faculty members gave to their participation in the training activities. Lastly, recommendations for further research and development in designing distance education are made.

METHODS

This project used a modified version of Dick and Carey's instructional systems design model.[12,13] The Systematic Instructional Design Model, promoted by Dick and Cary involves a systems approach that focuses on what the learner is expected to be able to do at the completion of instruction.[1] This approach connects the instructional strategy to the desired learning outcomes and provides linkages between each component in the model. The nine components of the Dick and Carey Model include: 1) goal identification, 2) instructional analysis, 3) learner and context analysis, 4) definition of objectives, 5) assessment instrument, 6) instructional strategy, 7) materials development, 8) formative evaluation, and 9) summative evaluation of instruction. By following this model in the development of the WebCLS course models, the teams were able to follow a consistent, development process.

Stage I: Analysis phase

Content expert teams (CETs) consisting of faculty members in the disciplines of clinical microbiology, clinical immunology, clinical chemistry, and clinical hematology respectively, worked together during the analysis phase of each course. Each team focused on their specific area of expertise and developed different courses. It was critical that discipline-specific faculty agreed on what content must be taught and how the course would be organized.

Stage 2: Design phase

At this point, the CETs divided their assigned course into modules in order to expedite the completion of courses. CET subgroups selected an appropriate instructional strategy to use and developed the actual content for a specific course module. As the module was being designed and developed, it was reviewed by the discipline specific CET and a consensus was obtained.

Stage 3: Development phase

During the development phase, the faculty subgroups worked with a development team to program the modules. A preliminary set of storyboards and flowcharts were created based on the instructional strategies operationalized during the design phase. A series of rapid prototypes were then created and tested to facilitate the development process using formative and usability testing techniques. Once an effective and efficient module had been completed, templates were generated to facilitate the development of the remaining modules. The prototypes were reviewed periodically by the CETs to ensure consensus with the content and tasks being presented. The prototypes were then programmed into actual on-line modules by the development team.

Stage 4: Implementation phase

Upon completion of the development of the course module, the module was pilot-tested by CLS and CLT students and laboratory practitioners. Modifications were made based on feedback from the pilot testing.

Faculty training

To prepare CLS faculty members for this extensive curriculum development effort, six training workshops were provided to give guidance in each phase's activities. The workshops were conducted by a SID expert. A faculty Website was established to support faculty participants between workshops. In addition, numerous teleconferences and small group meetings for module and project development were held over the course of the project.

Project evaluation

At the end of the three-year project period, data were collected from the workshop participants regarding their perceptions of the value of the SID process in developing their skills in Web-based, distance learning course development and teaching, and their skills in traditional course development and teaching.

A survey was developed in which the eight components of SID were listed and the respondents rated each component as to its value to them on a five-point Likert scale of 5 = very valuable to 1 = not very valuable. In addition to rating the value of each component of SID, the respondents were asked if they would like more training in any of the eight components.

The survey was sent via e-mail to 27 CLS faculty members who had participated in the training activities consistently over the entire period of the project. After three requests for responses, 24 participants completed the survey for an overall 89% response rate. Of the 24 respondents, 14 (58%) had participated in each of the SID workshops. An additional four respondents attended four of the six workshops. The responses of these 18 respondents (75%) were reported in this paper.

RESULTS

A majority of the respondents reported that all of the SID training components were valuable to them (Table 1). A strong majority of the respondents indicated that training in goal and instructional analyses (96%), media selection (94%), and aligning objectives, assessments, and instructional strategies (94%) were valuable to their distance education development and teaching skills.

In regard to the perceived value of the SID training to the participants' traditional course development and teaching skills, the percentages of respondents who indicated that the training was valuable were somewhat lower when compared to the value the respondents gave the training for distance education, but overall they were mostly positive. However, only 44% felt that the training they received in flowcharting, storyboarding, and rapid prototyping was valuable in improving their traditional course development skills. Specific training areas that faculty members found of value for traditional CLS teaching included the "flowcharting of instructional analysis to include all aspects of objective preparations and organization of order of subject matter presented to create flow of learning and reviewing" and the "study of teaching strategies to ensure presentation of material in a systematic strategy to ensure coverage of material and reinforcement and assessment (are) relate(d)."

Fewer respondents reported that training in flowcharting, storyboarding, and rapid prototyping was valuable in developing their distance education or traditional course development and teaching skills. However, a majority did indicate that they wanted more training in this area (Table 2). A majority of the respondents also indicated that they would like more training in alternative instructional strategies, media selection, and aligning objectives, assessments, and instructional strategies.

Table 1. Perceived value of SID training in developing respondents' distance education and traditional teaching skills

	Distance Education Skills				Traditional Teaching Skills			
Training Component	**Respondents***	**% Valuable†**	**Mean‡**	**S.D.**	**Respondents**	**% Valuable**	**Mean**	**S.D.**
Goal and instructional analyses	18	96	4.11	.82	17	84	4.12	.46
Context and learner analyses	17	83	3.94	.56	17	76	3.94	.66
Generating, clustering, and sequencing objectives	14	79	4.14	.77	16	94	4.06	.44
Alternative assessment methods, e.g., checklists/ portfolios	15	73	3.93	.70	16	81	4.66	.68
Alternative instructional strategies and grounded events	15	80	4.00	.85	16	81	3.86	.72
Media selection	15	94	4.20	.56	15	87	4.07	.59
Aligning objectives, assessments and instructional strategies	16	94	4.38	.62	17	94	4.24	.85
Flowcharting, storyboarding, and rapid prototyping	16	63	3.88	.81	16	44	3.19	.83

* Includes only those respondents who attended the training sessions (range of 14-18 respondents per component)

† Represents the combined percentages of respondents who indicated that the training component was very valuable or valuable to their course and teaching skills development.

‡ Represents the mean response to each training component on a 5 point scale where 5 = very valuable; 4 = valuable; 3 = uncertain; 2 = not valuable; and 1 = not very valuable.

Fifteen respondents (62%) reported that their participation in the WebCLS training activities improved their on campus (traditional) course development and teaching activities. Collectively, they reported that the training in SID helped them to: 1) move toward student-centered activities that included higher-level learning objectives; 2) organize their content to flow from learning objectives to teaching strategies to techniques to assess students' knowledge and skills; and 3) expand their teaching strategies to include both traditional lectures and Web-based instructional activities concurrently.

DISCUSSION

This project addressed the WBEC's report on the need for continuous and relevant training and support for faculty when developing Web-based course materials.[4] The SID sessions allowed the faculty to gain insight into good educational practices regarding Web-based education. Additionally, these sessions on instructional design gave faculty the tools to develop quality instructional units. The benefits to participating in the project included a systematic evaluation of the entire CLS curriculum and an opportunity to compare goals and objectives with other partners. The workshops also increased the Web-based development skills of the faculty and enhanced the quality of existing on-campus courses.

Participant comments indicated that they found that their courses were better aligned with the learning objectives and the anticipated outcomes, that the course organization improved with the use of flow-charting, and that they found alternative methods of delivering course material. One faculty member even found that sharing the systematic process with the students increased their learning of specific concepts.

Additionally, the participants found that they had increased the amount of student interactions, changing their teaching strategy from a lecture format to a more interactive format. Several faculty members indicated that they found ways to involve the students more in the learning process through on-line discussions and student centered learning activities. This outcome corresponds with Hirumi's hypothesis that key interactions must be planned for them to occur consistently throughout online learning.[10]

Table 2. Respondents' desire for more training in SID by training component (n=16)

Training Component	Yes responses	% of Total
Goal and instructional analyses	4	25
Context and learner analyses	5	31
Generating, clustering, and sequencing objectives	7	44
Alternative assessment methods, e.g., checklists and portfolio assessments	8	50
Alternative instructional strategies and grounded events	11	69
Media selection	11	69
Aligning objectives, assessments and instructional strategies	10	63
Flowcharting, storyboarding, and rapid prototyping	13	81

CONCLUSIONS

The data from the survey of CLS faculty must be considered qualitative and preliminary. Furthermore, there are limitations that must be considered: 1) the number of respondents to the survey was small and may not represent CLS faculty, generally; 2) as with most self-administered surveys, the chance of misinterpretation of survey items or inflation or deflation of perceptions is possible; and 3) the respondents were CLS faculty members who volunteered to participate, so they may have had some previous experience in or bias toward Web-based course development.

A strong majority of the faculty members who actively participated in the SID training valued their new skills in developing distance education courses, and improving their traditional teaching activities. Several respondents reported that they would like to have more training in specific components of the SID System. Additionally, the skills learned during the development of course materials in the WebCLS project were used by faculty members to improve their on-campus courses as well as with their Web-based courses. Based on these findings, further study using larger samples of CLS faculty members is recommended. Also, further study is warranted on the effect on student learning that could be attributed to the use of SID.

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REFERENCES

1. Dick W, Carey L, Carey J. The systematic design of instruction. 5th Ed. New York: Longman: Addison-Wesley Educational Publishers; 2001.
2. Mishra S. A design framework for online learning environments. Br J Educ Tech 2002;33(4):493-6.
3. Filipczak B. 1996. Training on intranets: the hope and the hype. Training 33 (9):24-32.
4. Kearsly G, Schneiderman B. Engagement theory: a framework for technology-based teaching and learning. Educ Tech 1996;38(5):20-3.
5. The Web-based Commission. The power of the Internet for learning: moving from promise to practice. August 25, 2000. http://interact.hpcnet.org/webcommission/doc.htm. Accessed August 6, 2005.
6. United States Department of Education, National Center for Education Statistics. Fast Response Survey System, Public School Teachers Use of Computers and the Internet, FRSS 70, Washington DC; 1999.
7. Green KC. 2001 October. Campus Computing Project. The 2001 National Survey of Information Technology in US Higher Education. http://www.campuscomputing.net. Accessed August 5, 2005.
8. Hirumi A. 2002 Summer. A framework for analyzing, designing, and sequencing planned elearning interactions. Quarterly Review of Distance Education 3(2):141-61.
9. Ely D. Toward a philosophy of instructional technology. Br J Educ Tech 1999;30(4):305-10.
10. Wilson BC. Adoption of learning technologies; toward new frameworks understanding the link between design and use. Educ Tech 1999;39(1):12-6.
11. Andrews DH, Goodson LA. A comparative analysis of models of instructional design. J Instructional Dev 1980;3(4):2-16.
12. Dick W, Carey L. The systematic design of instruction. New York: Harper Collins;1996.
13. Hirumi A. Training faculty in the systematic design of interactive distance education: a case study. Concurrent session held at the annual Association for Educational Communication and Technology conference. 1997 February; Albuquerque NM.

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From Single Cell Gene-based Diagnostics to Diagnostic Genomics: Current Applications and Future Perspectives

RICHARD ZHAO

Molecular diagnostics is a branch of clinical diagnostics that uses primarily DNA or RNA as a biomarker for clinical testing. It combines various gene-based amplification technologies with highly sophisticated detection methods for the clinical diagnosis of a vast variety of diseases including infectious diseases, cancer, and inherited diseases. The principal application of gene-based amplification technology is to identify pathogen or gene-specific nucleic acid sequences that are used as surrogate markers for the identification of either infectious pathogens or alteration of disease-related genes. There are generally three classes of gene-based amplification technologies: target-based, e.g., PCR; probe-based, e.g., LCR; and signal-based, e.g., bDNA. Real-time detection of PCR allows us to quantify amplified amplicons with a broad dynamic range and it offers a unique way to detect genetic mutations. Other technologies such as immuno-PCR and bio-barcode assay (BCA) combine different amplification tactics offering extreme detection sensitivity ranging from femtogram (10^{-15}) to zeptogram (10^{-21}). Even though quantum dots technology is in its infant stage, its potential to further increase diagnostic sensitivity and specificity is likely beyond our current imagination. Future diagnostic technologies include the use of genomic and proteomic approaches especially in pure cell types or even in the single-cell level, which open up endless new possibilities for gene-based diagnostics at entirely different levels. In this article, principles of various current gene-based amplification and detection technologies along with their clinical applications are discussed. New technologies that could potentially be used in future gene-based diagnosis are introduced.

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ABBREVIATIONS: BCA = bio-barcode amplification; bDNA = branched DNA; FRET = fluorescence resonance energy transfer; IPCR = immuno-PCR; LCM = laser capture microdissection; LCR = ligase chain reaction; NASBA = nucleic acid sequence based amplification; PCR = polymerase chain reaction; qdots = quantum dots; SDA = strand displacement array; SNP = single nucleotide polymorphism; T^M = melting temperature.

INDEX TERMS: gene-based amplification; molecular diagnostics.

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Focus Continuing Education Credit: *see pages 280 to 283 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES

1. Describe the classification of gene-based amplification methods.
2. State the principles of gene-based amplification methods.
3. Discuss the advantages and disadvantages of gene-based diagnostics.
4. Define future prospectives of gene-based diagnostics.

Twenty years has passed since the first description of the polymerase chain reaction (PCR) technique and its application to amplification of the β-globin gene sequence and to

restriction fragment length polymorphism analyses for the diagnosis of sickle-cell anemia.[1] During this period of time, there has been a nearly explosive growth in the number and variety of new gene-based methodologies in the field of gene-based diagnostics, also called molecular diagnostics.

Molecular diagnostics, based on nucleic acid amplification and detection technologies, is a branch of clinical diagnostics that uses primarily DNA or RNA originated either from patients or pathogens as the biomarker for the clinical testing. These gene-based diagnostic approaches span from detection of infectious diseases, cancer detection, and genetic diseases to forensic identity testing. One of the most desirable features of gene-based technologies in clinical diagnostics is their rapid, specific, and direct detection of genes of interest with very high sensitivity. Many of them allow additional and precise quantification of genes of interest with broad dynamic range. Importantly, they can be performed directly from clinical specimens without the need for laboratory cultivation, which is often time-consuming.

A unique feature of gene-based diagnosis of infectious diseases is that it can detect pathogens that cannot be detected by conventional diagnostic tests. For example, we can now readily detect many of the viral infections that are either difficult or impossible to culture in the laboratory. Another example of using gene-based assays is that we can now detect HIV infection in newborns on the day of birth. Previously, when antibody-based tests were used, a positive diagnosis was not possible until three to seven months of age. In another article in this Focus Section, Drs Niel Constantine and Richard Zhao from University of Maryland School of Medicine describe in detail the molecular-based diagnosis of HIV-1 infections.

Molecular testing can also be used to predict potential risk of an individual for onset of a specific cancer such as breast cancer with predispositions of *brca1/2* gene mutations.[2,3] Dr W Craig Hooper and his colleague Stacy C League from the Centers for Disease Control and Prevention provide a detailed description of this type of testing in congenital thrombosis in this issue. If clinical symptoms or other diagnostic tests implicate a specific cancer such as T-cell leukemia, gene-based testing could also be used to confirm diagnosis by detecting possible known T-cell gene arrangements. Furthermore, these same tests could be used to monitor success of anti-cancer therapy. For example, disappearance of the same T-cell gene re-arrangement would indicate cancer remission in the patient.

Molecular-based genetic tests can be used for many different purposes including carrier screening, prenatal testing, cancer risk, or confirmatory cancer testing, and genetic identification. Carrier testing normally involves identification of an individual who carries one copy of a gene for a disease that requires two copies for the disease to be expressed. An example of such a carrier testing includes Ashkenazi genetic carrier screening for an array of genetic diseases including Tay-Sachs diseases, Gaucher disease, cystic fibrosis, Canavan disease, Niemann-Pick, and familial dysautonomia. For further introduction on the use of molecular-based methodologies in genetic testing for cystic fibrosis, see the article in the next issue of *Clinical Laboratory Science* (Winter 2006) by Dr Edward Highsmith and his colleague Timothy S Uphoff from Mayo Clinic. Prenatal testing involves identification of gene alternations in the fetus for a specific genetic disease such as fragile X syndrome.

For cancer detection, molecular-based diagnosis has never been so close to reality in assisting physicians in detecting specific cancers or even in assisting in monitoring anti-cancer therapies. Dr Christopher Gocke from John Hopkins University provides specific information on molecular diagnosis and monitoring of hematological malignancies in the next issue (Winter 2006). Dr Gocke's article will provide detailed information on molecular diagnostics and monitoring of hematological malignancies.

Because of the genetic distinction of each individual, molecular-based testing can also be used for forensic or identity testing. Short tandem repeat (STR) assay has been used, for example, for paternity identification or criminal investigations. STR has also been implemented in the clinical setting to monitor efficacy of bone marrow transplantation of leukemia patients.

Altogether, gene-based molecular diagnostics provides us with tremendous power for clinical diagnosis of a broad range of diseases. With emergence of new and more powerful technologies, use of molecular diagnostics will only continue to grow. Implementation of more sensitive and specific tests with these new technologies in clinical diagnosis would allow us to continue improving patient care and thus saving more lives.

In this article, I will briefly describe principles of various gene-based amplification technologies including some of the most popular technologies such as Amplicor® (Roche), NucliSens® (Organon Teknika), Quantiplex® (Bayer), as well as real-time PCR methods that are currently available in the market for clinical diagnostics. Other technologies such as

LCR® (Abbott), Qâ-replicase® (Gene-Track), and SDA® (Becton Dickinson) will also be introduced. New emerging technologies such as immuno-PCR (IPCR), bio-barcode assay (BCA) and genomic/proteomic approaches, which have not yet been applied to clinical testing, but may prove to be even more powerful tools in molecular diagnostics, will be addressed.

Gene-based amplification typically consists of three major steps: primer-template hybridization, synthesis, and amplification. The gene-based amplification methodologies may be categorized based on the means of amplification, i.e., 1) target-based amplification methods, which are designed to detect and amplify the target gene of interest; 2) probe-based amplification methods, which rely on amplification of the probes that are homologous to a specific gene target, and, 3) signal-based technologies that amplify the signal rather than the gene target sequence.

GENE-BASED AMPLIFICATION TECHNOLOGIES

The gene-based amplification technologies can be further divided into PCR-based or non-PCR based methods. PCR-based method is an artificial DNA amplification method that is performed at various temperatures using a thermocycler. In contrast, most of the non-PCR methods take advantage of the natural nucleic acid amplification processes. For example, ligase chain reaction (LCR) mimics the enzymatic ligation process; nucleic acid sequence based amplification (NASBA) mimics viral RNA reverse transcription and transcription; strand displacement assay (SDA) resembles the DNA excision repair process; and Qβ-replicase RNA amplification resembles bacteriophage replication. Another common feature of non-PCR-based assays is that these assays can be carried out at constant temperature without thermocycling.

In theory, a single molecule can be detected and amplified to 10^9 to 10^{12} molecules within a few hours of the amplification reaction. In clinical practice, however, a much lower efficiency is obtained due to impurities and other intrinsic factors within the clinical specimens that may affect the efficiency of the amplification. Nevertheless, 10 to 100 copies of the target nucleic acid sequences, for example, can normally be detected with high probability.

DETECTION OF AMPLIFIED GENE PRODUCTS

Typically the amplified gene products can be detected by using various labeled reporter molecules such as enzymes, antigenic substrates, radioisotopes, chemiluminescent moieties, or fluorescent labels (Table 1). The classical means of gene detection is the use of DNA probes labeled with radioactive ^{32}P. Binding of the probe to the amplified product (hybridization) enables visualization of the gene product either by autoradiography or liquid scintillation counting. In most of the current commercially available diagnostic kits, however, radioactive isotopes are replaced by enzymes, affinity labels, chemiluminescent molecules, or fluorescent reporters for the detection of the amplified products. For example, direct labeling of oligonucleotides with the enzymes alkaline phosphatase or horseradish peroxidase, is responsible for the signal amplification in the bDNA signal amplification assay. Addition of enzyme specific substrates such as dioxetane to the amplified product gives light emission that can be detected by luminometry. Affinity labels such as biotin and digoxigenin are incorporated into the primers in PCR-based assays by enzymatic (nick translation or random-priming techniques) or non-enzymatic, e.g., photobiotin methods. Streptavidin linked to an enzyme is used to detect the moiety attached to the primers, and the detection is completed through the use of colorimetric or chemiluminescent substrates for the enzyme. Chemiluminescent markers are chemical groups that release light when exposed to certain substrates after the hybridization reaction is complete. The light emitted can be detected with X-ray film or a luminometer. Such assays have sensitivity ranges equal to or greater than those of ^{32}P.

Chemiluminescent labels, such as acridinium esters, have been incorporated into many commercially available probe kits and are used in a format that is

Table 1. Commonly used reporter molecules for detection of amplified gene products

Radioactive	^{32}P, ^{35}S, ^{3}H
Enzymatic	Alkaline phosphatase, horseradish peroxidase
Affinity labels	Biotin, digoxigenin
Chemiluminescent	Acridinium esters, sulfonamides
Fluorescent	FAM (SYBR Green I), JOE (VIC), TAMRA (NED or Cy3), ROX (Texas Red), and Cy5
Nanoparticles	Magnetic gold nanoparticles, quantum dots

rapid and highly efficient. Fluorescent labels have now been used in real-time PCR and single nucleotide polymorphism (SNP) analyses for the detection of amplified gene products or gene mutations.

There are five commonly used fluorophores including FAM (SYBR Green I), JOE (VIC), TAMRA (NED or Cy3), ROX (Texas Red), and Cy5. Each of them has a different excitation and emission spectrum thus allowing detection of single or multiplex (up to five colors) amplicons with great assay versatility.

Typically a fluorescent dye molecule is covalently attached to either a primer or a probe. Upon incorporation of the primer into an amplicon during gene-amplification in real-time PCR, increase of the fluorescence of the dye molecule can be detected at a single molecular level which thus allows real-time quantification of gene amplification cycle-by-cycle. The most recent new label for the detection of biological molecules is the fluorescent semiconductor nanocrystals, also known as quantum dots or qdots.[4] Evolved from electronic material science to the applications in molecular diagnostics, this new technology can monitor intracellular processes at the single molecular level with very high resolution. This extremely sensitive detecting label has far-reaching potential in improving sensitivity and specificity of future clinical diagnostic tests.

TARGET-BASED GENE AMPLIFICATION

Polymerase chain reaction (PCR)

PCR is a typical example of target-based amplification technology. For example, in the detection of a particular pathogen using PCR, a unique segment of DNA or RNA (rt-PCR), that represents a specific pathogen of interest, is selectively amplified and used to distinguish it from other organisms. Therefore, detection of a specific amplified gene sequence indicates the presence of a specific pathogen.

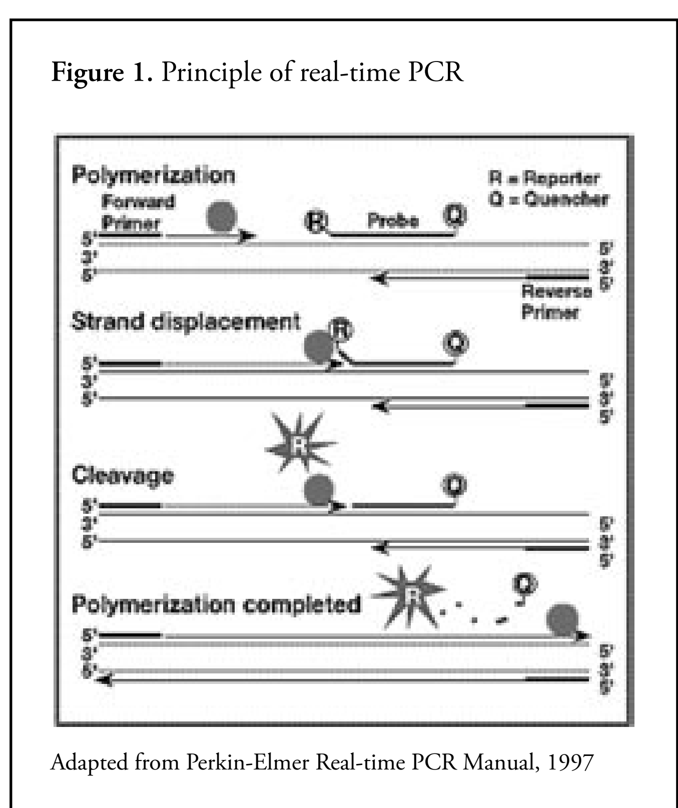
Protocols for detection of a vast variety of infective agents including viral, bacterial, fungal, and parasitic pathogens can be found in the literature. However, those 'in house' protocols are generally not adequate for immediate implementation in clinical testing unless, after proper assay validation, very restrictive QA/QC procedures are strictly enforced, and proper regulatory rules are followed. One limitation of the PCR-related techniques is that the blood sample has to be collected in tubes with anticoagulant such as ACD or EDTA. Heparin strongly inhibits the PCR reaction.

Cross-contamination is a major concern in PCR. However, an enzyme called uracil-N-glycosylase (UNG, commercially known as AmpErase®), which can break down pre-amplified amplicons, is incorporated in the Amplicor kits and works quite effectively in minimizing this potential problem. PCR-based technology for clinical diagnosis of various diseases is commercially available under the commercial name Amplicor™, through Roche Diagnostics Systems, Inc. These Amplicor tests include HIV, HCV, HBV, HPV, *Clamydia trachomatis,* and *Neisseria gonorrhea*.

Real-time PCR

PCR can also be used to quantify amplified gene sequences. However, the limitation associated with the traditional quantitative PCR is that the DNA copy numbers are calculated based on the final amplified gene products. Since DNA is amplified exponentially during PCR, a small variation in amplification efficiency early in the thermocycling process could potentially lead to a large variation in the final quantity of amplified products. Consequently, the intra- and inter-assay variability is often an issue to consider because the final accumulated product quantity may not necessarily be reflective of the initial copy number. Even though this limitation can be partially circumvented by adding an international quantification control, another shortcoming of the conventional PCR-based assays is that they generally have a narrow linear

Figure 1. Principle of real-time PCR



Adapted from Perkin-Elmer Real-time PCR Manual, 1997

dynamic range of approximately 3 logs due to the non-linear amplification of PCR. In contrast to conventional PCR, real-time PCR, which is also known as TaqMan™ or 5' exonuclease assay (Perkin-Elmer, Branchburg, NJ), quantifies PCR products cycle-by-cycle ('real-time') as they accumulate.[5] Unlike conventional PCR, an internal probe is added to the detection process, which is an oligonucleotide with both a fluorescent reporter and a fluorescent quencher dye attached. If a target sequence is present, the probe anneals between the forward and reverse primers and is then digested by the 5' nuclease activity of the DNA polymerase as PCR proceeds (Figure 1). Digestion of the probe DNA separates the reporter dye from the quencher dye, making the reporter dye signal detectable. Detection of the resulting fluorescence collectively provides an immediate real-time quantification of the PCR process.

There are several advantages to real-time PCR. It does not rely on the final product at end of the PCR amplification for quantification as regular PCR does. DNA copy numbers are determined based on the threshold cycle (C_t), which is directly proportional to the initial copy number. Thus, the measurement is highly accurate and reproducible. Since probe cleavage alone is responsible for increase of fluorescent signals, it is highly specific. For example, if the probe binds non-specifically to some other sequences that are not between the forward and reverse primers, it will not be cleaved or detected as part of the amplification. Conversely, non-specific primer binding and amplification will not be detected, because the probe is gene specific, hence does not bind to the same region. Since measurement of DNA copy number is directly proportional to the initial copy numbers, it allows much wider linear dynamic range of detection for quantification than conventional PCR. For an example of HIV-1 proviral DNA quantification using the real-time PCR technology, see reference.[6] Besides the quantificational power of real-time PCR, it can also be used for mutation detection or genotyping by using melting curve analysis of the amplified gene sequences. The rationale behind this capacity is based on the fact that every DNA fragment melts at a characteristic melting temperature (Tm). A mismatch between a mutated gene target and a fluorescent labeled wild type gene probe will result in a low Tm that can be readily detected by changes in fluorescent profile.

There are two types of probes used in real-time PCR: hydrolysis probes for real-time quantification and hybridization probes for single nucleotide polymorphisms (SNPs) and mutation detections. Examples of hydrolysis probes include TaqMan probes, molecular beacon probes, and scorpion primer-probes.[7,8] The TaqMan probes present as linear oligonucleotides; oligonucleotides of molecular beacon probes are typically in a hairpin format labeled at one end with a quencher and at the other end with a fluorescent reporter. In the absence of gene targets, the fluorescence is quenched. However, when molecular beacons hybridize to their gene targets, the hairpin structures open and they emit intense fluorescent signals for real-time detections. Scorpion primer-probes incorporate both the primer and probe into oligonucleotides that exist in a hairpin loop structure. Similar to molecular beacons, the fluorescence of scorpion primer-probes is normally quenched. Upon primer-mediated DNA synthesis of the gene targets, the scorpion probes hybridize to the newly formed complementary sequences, separating the fluorescent reporters from the quenchers thus restoring the fluorescence. In principle, molecular beacons and scorpion probes provide better probe-target binding specificity than linear probes.

Hybridization probes have been used for detections of mutations and SNPs by combined use of melting curve analysis and sequence-specific fluorescence resonance energy transfer (FRET). In a FRET melting curve analysis, two hybridization probes that are complementary to a continuous region of the gene target are labeled with fluorescein or LC Red 640, respectively. When the two fluorescent labeled probes come together upon gene-specific hybridization, excitation of fluorescein causes an energy transfer, which subsequently results in excitation of the LC Red 640 molecule. Such a FRET allows detection of specific probe-gene hybridization. Melting curve analysis is based on the fact that each double strand DNA has its own unique melting temperature (Tm). A single change in nucleotide will result in change of Tm. Therefore, combined use of FRET for gene-specific hybridization with mutation-specific changes of Tm enables detection of specific gene mutations with high accuracy.

Nucleic acid sequence based amplification (NASBA)

NASBA is another target-based amplification method that is formerly known as 3SR (self-sustaining sequence replication). In contrast to PCR, however, this method is essentially an in vitro version of the natural replication of retroviral RNA. Therefore, this assay is useful only for detecting RNA targets such as HIV or HCV. A standard NASBA reaction contains T7 RNA polymerase, RNase H, avian myeloblastosis virus (AMV) reverse transcriptase, nucleoside triphosphates, two specific primers, and appropriate buffer contents. For quantification of HIV viral load, for example, primer 1 is about

45 bases in length with an average of 20 bases at the 3' end that are complementary to the 3' side of the target sequence. The 5' end of this primer contains a promoter sequence that is recognized by T7 RNA polymerase. Primer 2 is about 20 bases in length and is derived from the opposite (5' direction) side of the target sequence. The NASBA test involves repetitive reverse transcription from an RNA template by AMV reverse transcriptase and RNA amplification from cDNA by gene transcription via T7 promoter. Approximately 40 copies of RNA can be made (versus two copies/cycle in PCR) in each cycle for each copy of the RNA target. Within a 90 minute reaction, approximately 10^{12} RNA molecules can be made starting with 10 copies of purified RNA molecules.

One advantage of using NASBA is that it can use any types of specimen, e.g., serum, plasma, cerebrospinal fluid, tissue, seminal plasma, or genital secretions. There is no specific requirement for anticoagulant. A drawback of this test is that it requires a separate RNA extraction step, making it more labor intensive. Consequently, a potential problem with NASBA is the significant risk of carry-over contamination between samples since much pipetting and tube opening must be performed for the isolation of viral RNA from plasma or serum specimens. The commercial name of NASBA amplification products is NucliSens™ through bioMerieux, Inc. Commercial tests currently are available include NucliSens CMV pp67, RSV A+B, Enterovirus, and HIV-1.

PROBE-BASED AMPLIFICATION

Ligase chain reaction (LCR)

In contrast to target-based amplification technology, probe-based technique is designed to amplify the probe homologous to the target. LCR is a method designed to amplify the probes that are homologous to a specific target of interest. The rationale behind this method is that ligation is most efficient when two discrete probes hybridize to the target in a head-to-tail fashion. Once the first pair of probes is ligated, it can in turn serve as a template for annealing and ligation of a second pair of probes complementary to its sequence. Recycled ligations between these two sets of ligated probes plus the original targets will result in a logarithmic accumulation of ligation products. Diagnostic tests using LCR are developed under the trademark name of LCx at Abbott Laboratories. A HIV-1 LCx commercial test is available outside the United States.

Strand displacement amplification (SDA)

The key principle of SDA resembles DNA excision DNA repair.[9] In the presence of a single strand nick on a double stranded DNA molecule, the nicked strand will be prejudicially displaced by a newly synthesized DNA strand. If it were possible to create a nick repeatedly at the same location, displaced DNA strands of the same size could then be produced continuously. To accomplish this goal, an endonuclease restriction enzyme *Hinc*II or *BsoB*I is used, which only nicks DNA on one strand of its recognition site (5'-GTTGAC-3') when the opposite strand is hemiphosphorothiolated (DNA contains dATPαS instead of dATP) during DNA synthesis. The primers used in this method contain this particular recognition site and are designed in such a way that the size of the displaced strand will be the same as the target. Therefore, the displaced DNA strands can also be used as templates for subsequent reactions. Repetition of this nicking and displacement cycle will result in a geometric accumulation of the synthetic product with approximately a 10^8-fold amplification within two hours. Although this method has the potential to be used in diagnosis of many diseases such as HIV-1 and *Mycobacterium spp*, no commercial tests have yet been marketed.

Qβ-replicase amplified probe assay

Qβ-replicase amplified probe assay is another probe-based amplification method. This method takes advantage of a unique feature of the bacteriophage Qβ-replicase, which replicates only those RNA substrates with a specific secondary structure. Therefore, by incorporating a target-specific probe into a Qβ-replicase substrate, not only does this probe-containing substrate hybridize to the gene target, but the Qβ–replicase specifically amplifies only the hybridized RNA substrates. Unhybridized probes will be removed upon RNaseIII treatment. Using this method, one copy of the target could be amplified into 10^9 copies with 30 minute incubation. The use of Qβ-replicase amplified probe assay has been described previously by Gene-Track Inc. for the detection of HIV, *C. trachomatis*, *M. avium*, and *M. tuberculosis*. However, no commercial kits are yet available using this technology.

SIGNAL-BASED AMPLIFICATION

Branched DNA (bDNA)

The bDNA method is a probe-based amplification assay developed by Chiron Diagnostics and currently owned by Bayer Inc. It may be used to detect both DNA and RNA targets. Several hybridization steps are incorporated in this method. After DNA or RNA extraction and denaturation of the target, the first hybridization step is to position the target using a capture probe attached to a solid surface. Once the target is captured, a second probe (extender probe) is used to hybridize to the target at an adjacent sequence in relation to the first hybridizing region. An

amplification multimer (branched DNA) which is homologous to the extender probe will then attach to the extender. Alkaline phosphatase-labeled oligonucleotides will stick to these branches with 3,000 to 22,380 branch sites per target molecule. This branched tree type complex can be detected using chemiluminescence. This technique is advantageous in that the assay is simple to perform with a low inherent variability and limited carry-over contamination. Although a larger number of assays can be performed per day, the turn around time is significantly longer than PCR-based assays. This may be an important factor in situations where results are required quickly. The bDNA-based diagnostic tests are available under the trade marker of Quantiplex™ or Versant® through Bayer Inc. for the detection of various infections including HIV, HBV, HCV, and CMV.

OTHER GENE AMPLIFICATION TECHNOLOGIES

There are a number of new emerging technologies that have not yet been applied to clinical testing, but may prove to be very useful tools in future gene diagnostics. Only two particular technologies are discussed in the following.

Immuno-PCR (IPCR)

Immuno-PCR is a new emerging technique that combines the versatility of the well-established ELISA methodology with the amplification power of real-time PCR.[10,11] However, it detects proteins instead of nucleic acids. For example, IPCR has been used for quantification of HIV-1 p24 antigenemia.[12] In this assay, signal DNA molecules are attached to the anti-p24 antibody. While p24 antigens are detected by anti-p24 antibody using ELISA, the number of bound antigen-antibody complexes is quantified by real-time PCR amplification of the signal DNA molecules. Testing of patient samples by using this assay detected p24 antigen at the femtogram (fg) level, which is at least 10^3 more sensitive than conventional ELISA assays. Although IPCR could in principle become one of the most sensitive diagnostic tools, it is technically difficult to create the antibody-DNA conjugates. There are also increasing concerns about the substantial backgrounds it generates due to nonspecific binding of the antibody-DNA conjugates to the solid phases. Thus the actual utility of this methodology in clinical diagnostics is yet to be seen.

Bio-barcode assay (BCA)

Nanotechnology is making another wave of revolution in the diagnostics industry.[13] The combination of nanotechnology and diagnostic biology creates an unprecedented and innovative way to detect biological molecules at an extreme low level, providing the potential for a generation of a variety of new and much more sensitive diagnostic tests. One of the nanotechnology-based techniques, known as bio-barcode amplification (BCA), can potentially detect multiple disease targets in a single clinical sample simultaneously.[14] This new technology detects both protein and nucleic acids and is based on capture of protein or nucleic acids by magnetic gold nanoparticles.[15] For the detection of a DNA gene target, for example, each magnetic gold nanoparticle (approximately 30 nanometers in diameter) is attached to hundreds to thousands of gene-specific oligonucleotides. These gene-specific nanoparticles can capture the gene of interest by sandwiching the DNA between two particles. The 'particle-DNA-particle' sandwich is then removed magnetically for final DNA recognition and quantification. Since each nanoparticle is coated with thousands of signal oligonucleotides, it thus automatically amplifies each of the DNA molecules by at least a thousand-fold. Because the DNA molecules are collected by magnetic enrichment, the BCA offers extreme high detection sensitivity with its limit at approximately 500 zeptomolar (zM) target DNA, i.e., less than 500 molecules per milliliter of solution.[14] BCA has also been tested in the detection of amyloid-β-derived diffusible ligands (ADDLs), a protein marker that can be used to diagnose onset of Alzheimer's disease.[16] Normally the ADDL concentration in brain or CSF is too low to be detected (<1 pM) by conventional diagnostic methods. However, ADDL concentrations were consistently detected by BCA and shown to be higher in the patients with Alzheimer's disease than nondemented age-matched controls.[16] Therefore, if BCA were validated in further clinical trials, this assay could become the first diagnostic test to conclusively diagnose Alzheimer's disease in living patients.

Genomics and proteomics in molecular diagnostics

Development of microarray technology now enables us to scan genome-wide genetic changes at the DNA, RNA, or protein levels. For example, DNA sequencing arrays are designed for simultaneous determination of multiple nucleotide sequences of interest. This approach is suitable for SNP detection of multiple genes, which could allow us to detect multiple mutations that contribute to a specific disease or predisposition of multiple diseases. Expression arrays are used for detection of changes at the gene transcriptional levels such as mRNA expressed in a tissue. Since tumorgenesis is typically the result of accumulating changes of gene expression or mutations, persistent changes in gene expression levels such as activation of oncogenes or loss of tumor suppressor gene expressions could be a strong indication of onset of cancerous cellular growth. Golub and colleagues were among the first to use gene expression profiling to distinguish acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL).[17] Similarly, comparison of protein abundance or searching for subsets of proteins linking to a specific disease

(protein profiling) at the genome-wide scale offers additional power to evaluate potential pathological changes in a patient, e.g., onset of cancer. Typically, combined use of multiple techniques is needed to decipher the complex nature of these datasets generated by genomic or proteomic methods. For example, to identify protein biomarkers that link to a specific disease, combined use of artificial-intelligence-based informatic algorithms for data mining and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for protein identification are needed. An increasing number of studies that utilize these strategies to identify subsets of genes or proteins as biomarkers for variety of cancers are available, such as melanoma, ovarian cancer, oral cancer, and colorectal cancer.[18-21] Regardless of their high potential in molecular diagnostics, the use of genomic and proteomic biomarkers for earlier detection and risk assessment of genetic diseases has not reached the level of confidence required for clinical practice. There is an ongoing nation-wide effort coordinated by the National Cancer Institute's Early Detection Research Network (EDRN); its specific goal is to identify, validate, and implement specific cancer biomarkers for the earlier detection and risk assessment of cancers by using various genomic and proteomic approaches. It is hopeful that some of the future discoveries will soon lead to diagnostic tests that can be used in the clinic setting.

Single-cell diagnostics

Current diagnostic specimens such as blood or biopsy samples normally contain a mixture of cell types. Because many diseases such as cancers may initiate from a single cell type or even start with a single cellular event, early detection of cancer or monitoring anti-cancer therapy requires precise molecular diagnostics at the level of pure cell population or possibly in a single cell. However, procurement of a pure cell population from clinical specimens has been hampered by the lack of suitable technical tools. Development of the laser capture microdissection (LCM) technique now enables us to isolate a specific cell subtype or even a single cell under the microscope.[22] Thus combining the analytic power of molecular-based testing and the LCM cell isolation technique, molecular diagnostics is now elevated to a whole different level. For example, by using the single-cell complementary DNA array and LCM, we are now capable of analyzing gene or protein profiles of two adjacent cell types within a single tissue such as a tumor. Even though molecular diagnosis at the single-cell level is not yet a reality in the clinical setting, analysis of single-cell genetic changes using the proven techniques should provide insights into the molecular pathology underlying many of the human diseases and thus promises more precise disease diagnosis. For more detailed reviews of this subject, see references.[23,24]

SUMMARY

In the last two decades, we have experienced explosive growth in the number and variety of new gene-based amplification and detection technologies, revolutionizing our ways of conducting clinical diagnosis and practicing medicine. However, as with any other new technologies, gene-based amplification and detection technologies have their own strengths and pitfalls. The obvious advantages of these technologies are that they are rapid, specific, and highly sensitive, involving direct detection from clinical specimens and avoiding the need for laboratory cultivation. As a result, the turn-around time is rapid. Even though the cost of these tests on a per test basis is relatively high at present, the quick turn-around time allows timely treatment for many life-threatening diseases. This minimizes the time of hospital stay and therefore reduces the overall cost.

The shortcomings of these new technologies, however, are potential false positives due to carry-over contamination or false negatives due to inhibitory effect on gene amplification. These potential technical errors could have significant impact on clinical care. Despite the obvious advantages and power the new gene diagnostic techniques can offer us, it would be a mistake to assume that they can replace the conventional diagnostic techniques. Rather than replacing the conventional methods of diagnostic testing such as classical microbiological methods, gene-based amplification technologies should serve as an adjunct to those methods and as a more rapid and less cumbersome alternative for clinical diagnosis. Overall, these new gene-based diagnostic technologies will undoubtedly significantly expand and expedite our ability in daily clinical diagnostics. Future implementation of genomic and proteomic approaches in clinical testing especially in pure cell type or at the single-cell level will undoubtedly expand our repertoires in clinical testing. Some of the current and future new diagnostic techniques will eventually become mainstream tools in clinical diagnostics and may very well replace some conventional diagnostic methods.

REFERENCES

1. Saiki RK, Scharf S, Faloona F, and others. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Sci 1985;230:1350-4.
2. Miki Y, Swensen J, Shattuck-Eidens D, and others. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Sci 1994;266:66-71.
3. Marx J. A second breast cancer susceptibility gene is found. Sci 1996;271:30-1.
4. Michalet X, Pinaud FF, Bentolila LA, and others. Quantum dots for live cells, in vivo imaging, and diagnostics. Sci 2005;307:538-44.

5. Holland P, Abramso R, Watson R, Gelfand D. Detection of specific polymerase chain reaction product by utilizating the 5' - 3' exonuclease activity of *Thermus aquaticus* DNA polymerase. Proc Natl Acad Sci U S A. 1991;88:7276-80.
6. Zhao Y, Yu M, Miller JW, and others. Quantification of human immunodeficiency virus type 1 proviral DNA by using TaqMan technology. J Clin Microbiol 2002;40:675-8.
7. Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol. 1996;14:303-8.
8. Thelwell N, Millington S, Solinas A, and others. Mode of action and application of Scorpion primers to mutation detection. Nucleic Acids Res. 2000;28:3752-61.
9. Walker GT, Fraiser MS, Schram JL, and others. Strand displacement amplification–an isothermal, in vitro DNA amplification technique. Nucleic Acids Res 1992;20:1691-6.
10. Sano T, Smith CL, Cantor CR. Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. Sci 1992;258:120-2.
11. Niemeyer CM, Adler M, Blohm D. Fluorometric polymerase chain reaction (PCR) enzyme-linked immunosorbent assay for quantification of immuno-PCR products in microplates. Anal Biochem. 1997;246:140-5.
12. Barletta JM, Edelman DC, Constantine NT. Lowering the detection limits of HIV-1 viral load using real-time immuno-PCR for HIV-1 p24 antigen. Am J Clin Pathol. 2004;122:20-7.
13. Vo-Dinh T. Nanobiosensors: probing the sanctuary of individual living cells. J Cell Biochem Suppl. 2002;39:154-61.
14. Nam JM, Stoeva SI, Mirkin CA. Bio-bar-code-based DNA detection with PCR-like sensitivity. J Am Chem Soc. 2004;126:5932-3.
15. Taton TA, Mirkin CA, Letsinger RL. Scanometric DNA array detection with nanoparticle probes. Sci 2000;289:1757-60.
16. Georganopoulou DG, Chang L, Nam JM, and others. Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. Proc Natl Acad Sci U S A. 2005;102:2273-6.
17. Golub TR, Slonim DK, Tamayo P, and others. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Sci 1999;286:531-7.
18. Clark EA, Golub TR, Lander ES, Hynes RO. Genomic analysis of metastasis reveals an essential role for RhoC. Nature. 2000;406:532-5.
19. Ismail RS, Baldwin RL, Fang J, and others. Differential gene expression between normal and tumor-derived ovarian epithelial cells. Cancer Res. 2000;60:6744-9.
20. Alevizos I, Mahadevappa M, Zhang X, and others. Oral cancer in vivo gene expression profiling assisted by laser capture microdissection and microarray analysis. Oncogene. 2001;20:6196-204.
21. Hegde P, Qi R, Gaspard R, and others. Identification of tumor markers in models of human colorectal cancer using a 19,200-element complementary DNA microarray. Cancer Res. 2001;61:7792-7.
22. Emmert-Buck MR, Bonner RF, Smith PD, and others. Laser capture microdissection. Sci 1996;274:998-1001.
23. Player A, Barrett JC, Kawasaki ES. Laser capture microdissection, microarrays and the precise definition of a cancer cell. Expert Rev Mol Diagn 2004;4:831-40.
24. Kawasaki ES. Microarrays and the gene expression profile of a single cell. Ann N Y Acad Sci 2004;1020:92-100.

2006 Annual Meeting Abstract Deadline

The deadline for abstracts for oral or poster presentations of research or case studies at the 2006 ASCLS Annual Meeting is January 15, 2006. Submission instructions and the proposal form may be found at www.ascls.org/conferences. The completed proposal form and abstract must be submitted electronically by the deadline.

The 2006 Annual Meeting will be held July 25-29 in Chicago. Additional meeting information will be available at the ASCLS Conferences website.

Molecular-based Laboratory Testing and Monitoring for Human Immunodeficiency Virus Infections

NIEL CONSTANTINE, RICHARD ZHAO

Applications of laboratory testing for human immunodeficiency virus type 1 (HIV-1) infection have made significant impact on clinical care of HIV-infected patients globally. As these technologies continue to evolve and new technologies emerge, unique and highly sensitive nucleic acid-based testing methods will offer more and better means for us to guide physicians in anti-retroviral treatment strategies and clinical management of HIV infected patients. In this review we discuss a variety of current molecular-based methods that are available for HIV testing including diagnosis, monitoring disease progression, and detection of drug resistance to anti-retroviral therapy. Newer approaches that could be used in future HIV testing are also introduced.

ABBREVIATIONS: bDNA = branched DNA; IPCR = immuno-PCR; NASBA = nucleic acid sequence based amplification; NAT = nucleic acid tests; PCR = polymerase chain reaction.

INDEX TERMS: diagnosis; disease progression; drug resistance; HIV infection; monitoring.

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LEARNING OBJECTIVES

1. Describe the uses of molecular-based assays in addressing issues related to HIV infection.
2. List three specific molecular methods commonly used to quantify HIV viral load and describe how they differ in principle.
3. Describe molecular methods that can be used to determine viral resistance to anti-retroviral drugs.

Methods for detection of viral nucleic acids (RNA or DNA) using nucleic acid tests (NATs) grew from a mere research tool to a clinically useful tool in the mid 1990s. Within a few years, molecular tests were being used to detect viral RNA for screening of blood and blood products.[1] Subsequently they were implemented in the clinical laboratories for use in the management of infected individuals and prognosis of disease outcome. More recently, NATs have been further modified to allow genotyping of viral types, groups, and clades. They can be additionally used to determine gene mutations associated with viral resistance to anti-retroviral therapy. They are now becoming indispensable tools in the clinical setting for assisting physicians and practitioners in their successful management of persons with HIV/AIDS. For example, the development of highly active antiretroviral therapy (HAART) has heralded marked advances in understanding the dynamic equilibrium of HIV-1 replication, cell destruction, and cell replenishment. The rapid reduction in virus levels a few weeks after initiation of HAART has led to dramatic improvements in the clinical management of HIV-infected patients. However, many problems remain mainly because of the emergence of viral resistance to drug therapy, the realization of the existence of viral genomes

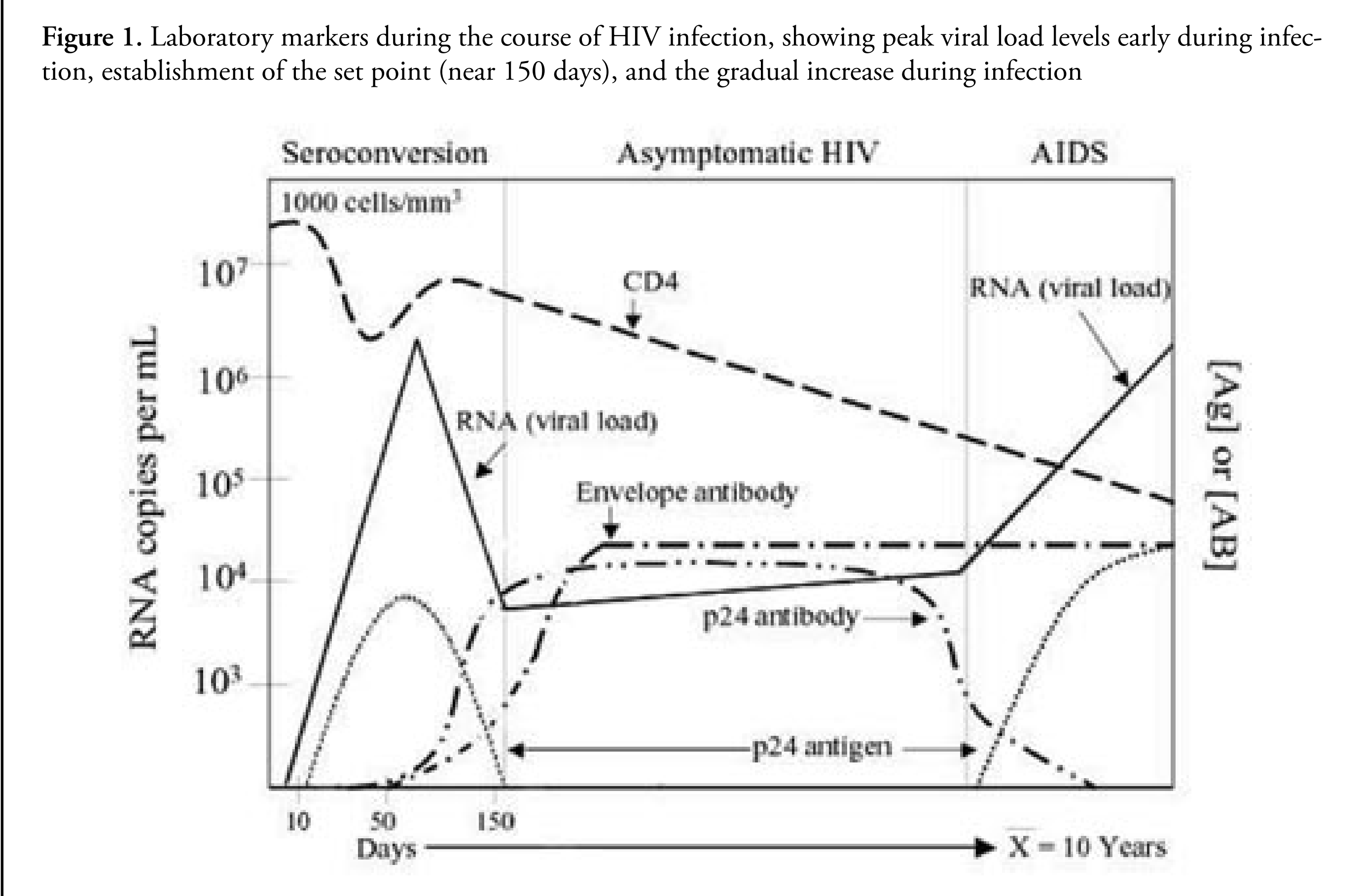
integrated in chromosomes (viral latency), and an understanding of the distribution of virus in other cellular compartments, e.g., lymphatic tissue. The use of NATs for quantification of viral burden offers a powerful means for us to: 1) measure the viral load baseline prior to initiation of HAART, 2) assess the efficacy of initiation of antiviral drug treatment, 3) detect the onset of drug resistance both after initial therapy and after prolonged therapy, and 4) predict the development of AIDS-defining opportunistic infections and disease progression. Thus, molecular diagnostics has dramatically changed the way HIV related medicine is conducted, and has not only helped to prevent the spread of infection but has resulted in an extension of the length and quality of life. This brief review provides an update on some of the major NATs that are available and their appropriate usage for addressing HIV infection.

USES OF MOLECULAR METHODS FOR HIV DIAGNOSIS

NATs can be used to resolve the infection status of individuals with indeterminate serological results. They are especially valuable in identifying HIV infection in newborns from seropositive mothers. NATs such as DNA or RNA PCR now allow us to diagnose HIV infection soon after birth instead of waiting at least three to seven months for a final diagnosis, thus enabling physicians to treat only those babies with infections. Unlike adults, serological testing of children under the age of 18 months has little diagnostic value because of the presence of maternal antibody in the newborn.[2]

In most individuals, the HIV seronegative window period of about three weeks following initial infection can be shortened to about 12 days by using molecular DNA or RNA tests.[3] Viral RNA is the earliest marker appearing after infection and follows a predictable course with RNA levels 'ramping up' during the first five to six weeks to reach a peak of nearly one million copies/mL within two to six months; it subsequently falls (without treatment) 2 to 3 logs to a 'set point' of about 10,000 copies/mL.[4] The ramping up period represents the doubling rates of HIV (21.5 hours), and about one billion virions are produced each day, regardless of the stage of infection. From

Figure 1. Laboratory markers during the course of HIV infection, showing peak viral load levels early during infection, establishment of the set point (near 150 days), and the gradual increase during infection



the set point, viral load remains at fairly constant levels (low to moderate) for up to ten years before rising to high levels when AIDS occurs. It is the immune response that combats the viremia and keeps the viral levels in the blood fairly constant. The set point appears to dictate the future course of the disease, with higher set points signaling a poor prognosis for the patients. For example, infected persons whose HIV RNA viral load levels exceed 100,000 copies/mL at six months after infection are 10-fold more likely to progress to AIDS within five years than those with levels less than 100,000 copies/mL.[5] Figure 1 depicts the relationship of RNA levels to other laboratory markers during the course of HIV infection.

MONITORING RESPONSE TO THERAPY AND DISEASE PROGRESSION

Monitoring viral burden has become essential to determine when to institute drug therapy and when therapy may need to be changed. The use of NATs has proven to be the most important means to assist in the management of patients because NATs yield insight into the degree of the viral burden. Experts suggest to measure viral load twice during a two-week interval to determine the viral baseline. The same tests should be followed by a single test every several months thereafter to determine disease progression.[6] Typical response of a drug naïve patient after initiation of antiviral treatment should be expected to be 0.7 to 0.8 log viral reduction using reverse transcriptase inhibitors or 2-3 logs viral reduction with protease inhibitors. Combined use of RT and protease inhibitors often results in significant reduction in viral load to a non-detectable level by the current molecular methods, i.e., less than 50 viral RNA copies/mL.[7] Clinical data further suggest that early treatment in acutely infected patients may provide additional benefit.[8,9]

There are three commercial test kits that are FDA-approved for the measurement of HIV-1 RNA (viral load) in plasma: 1)

Table 1. Comparison of three commonly used commercial methods in determination of HIV-1 viral load

METHOD	**RT-PCR**	**NASBA**	**bDNA**
Test name (Manufacturer)	Amplicor (Roche)	NucliSens (Organon Teknika)	Versant (Bayer)
Lower detection limit	400 copies/mL (standard) 50 copies/mL (ultrasensitive)	40-400 copies/mL 40 (but not consistently)	500 copies/mL (2.0) 50 copies/mL (3.0)
Linear detection range (copies/mL)	400 – 750,000 (standard) 50 – 100,000 (ultrasensitive)	40 – 10,000,000	500 – 1,600,000 (2.0) 50 – 500,000 (3.0)
Volume of plasma required	0.2 mL (standard) 0.5 mL (ultrasensitive)	0.01 to 2.0 mL	1.0 mL
Coeffieient of variation (%)	<30	≤29	≤20
Estimated time to perform test	9 hours (standard) 10 hours (ultrasensitive)	5 hours	36 hours
Specimen type	Plasma (EDTA or ACD)	PBMC, whole blood or any body fluid	Plasma (EDTA or ACD)
Maximum number of tests per run	21 to 45	20	80 to 120
Estimated cost of capital equipment	$20,000	$10,000	$40,000 to $45,000

two versions of the Amplicor Monitor™ (Roche Diagnostics Systems) which is a RT-PCR method, 2) the Versant™ HIV-1 RNA 3.0 kit (Bayer Inc) which is known as bDNA, and 3) the NucliSen™ HIV-1 RNA QT System (bioMerieux Inc), which is also known as nucleic acid sequence based amplification (NASBA). The Amplicor Monitor RT-PCR based testing system is a target-based amplification assay based on the principle of RT-PCR, i.e., the viral RNA is first reverse transcribed by reverse transcriptase into cDNA and the DNA is then amplified by PCR. Similarly, the NucliSen HIV-1 RNA QT assay is a target-based amplification process, but it directly amplifies the viral RNA using a method mimicking natural replication of retroviruses.[10] In contrast, the Versant HIV RNA 3.0, formally known as the Quantiplex™ or bDNA assay (Chiron), uses a signal-based amplification principle.[11] All three commercial methods, described in more detail below, achieve very similar results for the quantification of HIV-1 RNA (Table 1).[12]

Amplicor Monitor

The Amplicor Monitor assay utilizes a template-based RT-PCR amplification process to convert the target viral RNA template to a DNA form that can be amplified and detected, i.e., the method combines reverse transcription and DNA amplification reactions. The enzyme reverse transcriptase (RT) converts viral RNA into cDNA which is amplified using PCR. By incorporating a specific RT (*rTth* polymerase isolated from a thermophilic bacterium, *Thermus thermophilus*), a one step assay is possible because *rTth* polymerase can carry out both reverse transcription and DNA amplification.

The RT-PCR reaction is carried out in an instrument called a thermocycler that allows for the rapid changes in temperature required for cycling through extension and denaturation steps. Oligonucleotide primers that are biotin-labeled, initiate the replication of DNA and the DNA sequence products (amplicons) become biotinylated. These products are subsequently captured by complementary oligonucleotides immobilized on a microtiter plate. The products are detected by an avidin-horseradish peroxidase conjugate that binds to the biotinylated captured amplicons; a substrate is subsequently added to form color, and a microtiter plate spectrophotometer is used to measure the level of signal (color) generated by the amplified products.

An automated system called the COBAS Ampliscreen is also available for HIV RNA testing and is approved by the FDA; it offers full automation for the RT-PCR method. Technically, the RT-PCR assay is moderately difficult to learn and requires a skilled user; a relatively slow learning curve should be expected.

Cross contamination by amplicons is a potential problem for PCR-based assay. To prevent cross contamination, an enzyme, uracil-N-glycosylase (UNG, commercially known as AmpErase®), is included within the Amplicor reaction to eliminate previously amplified amplicons, in which deoxyuridine triphosphates (dUTP) are intentionally incorporated into DNA. Furthermore, a uni-directional work flow is strongly recommended in order to maintain a low risk of cross contamination when performing PCR.

Nucleic acid based sequence amplification

Similar to the Amplicor Monitor assay, the NASBA assay is a target-based amplification method.[14] In essence, it parallels the natural replication of retroviral RNA in vitro. With the combined use of avian myeloblastosis virus (AMV) reverse transcriptase, T7 RNA polymerase, and RNase H in one tube, the NASBA test involves repetitive steps of reverse transcription from the viral RNA template with subsequent RNA amplification from cDNA. Approximately 40 copies of RNA can be made per cycle for each copy of the RNA target, and within a 90 minute reaction; approximately 1,000 RNA molecules can be made from 10 starting copies of purified RNA molecules. The amplification process results in the production of large quantities (billions) of the target sequence of single-stranded, anti-sense RNA that is complementary to the original target RNA.

The amplified single-stranded RNA product is detected by hybridization to complementary wild-type or calibrator-specific ruthenium-labeled oligonucleotide probes tagged with an electrochemiluminescence moiety that is subsequently coupled to paramagnetic beads. The amount of chemiluminescence is proportional to the quantity of the amplified product.

Three internal controls are built into this reaction to monitor the efficiency of amplification and to calculate the viral load. An advantage to this method is that all the reaction steps are conducted at constant (isothermal) temperature (40 °C to 41 °C). A disadvantage is that a semi-automated electrochemiluminescence (ECL) detection instrument is required to measure the amplified products. Another advantage of using the NASBA system is that non-plasma samples can be used. The method requires a separate RNA extraction step, making it more labor intensive, although new pre-amplification procedures (NucliSens extractor) using silica for extraction are rapid and efficient.

bDNA assay

Unlike the Amplicor and NASBA assays that amplify the target viral nucleic acid, the bDNA technique amplifies the signal from a captured viral RNA target by sequential oligonucleotide hybridization steps.[11] After disrupting the virion and denaturing the target, probes directed toward the *pol* gene hybridize to the target sequences. A mixture of capture probes attached to a solid surface of a microtiter plate bind the target. Once the viral target is captured, amplifier and preamplifier extender probes are used to hybridize to the target at an adjacent sequence region, resulting in a branched DNA complex. Alkaline phosphatase-labeled oligonucleotides attach to these branches with approximately 3,000 to 22,380 branch sites per target molecule. This branched tree type complex is detected using chemiluminescence. The technique has advantages in that the assay is simple to perform with a low inherent variability and limited carry-over contamination. Also, because of the many DNA probes, it recognizes a broad spectrum of HIV sequences, including those of viral variants, and does not require viral RNA purification (virions are concentrated by centrifugation). Although a larger number of assays can be performed per day, the turn around time for results is significantly longer than the other assays. This may be an important factor in situations where rapid turnaround times are required. The detection limit has recently been reduced to 50 copies/mL in the 3.0 version.

The requirement of larger volumes of plasma may be a concern when testing infants and newborns. A pediatric assay that requires only 50 µL of plasma is available through Chiron. However, the assay design was based on the first generation of bDNA method, thus the detection limit is too high.[15]

Other methods to measure viral load

In addition to these three commercial viral load assays, a number of other methods are used internationally or are under development. These include the ligase chain reaction (LCR, Abbott Laboratories), Qβ-replicase (Gene Track Inc.), strand displacement amplification (SDA, Becton Dickinson Co), hybrid capture system (Digene Co.), transcription mediated amplification (TMA, Gen-Probe Inc), and a quantitative method to measure the enzyme reverse transcriptase (ExaVir Load, Cavidi Tech). Each is based on different scientific principles, but they all have potential in providing similar and competitive assays for measuring HIV-1 viral load.

The Cavidi Tech RT assay is unique in that it is an ELISA-based assay. It incorporates determination of reverse transcriptase enzymatic activity and quantification of revised transcribed cDNA for viral load determination.[3] Similar to an EIA method, the method requires two to three days for completion. Our laboratory recently reported on the method of immuno-PCR for the detection of ultra low levels of HIV p24 antigen.[16] In this technique, a serologic antigen capture EIA method is coupled to real-time PCR where the detector antibody is indirectly labeled with a 500 base pair strand of DNA. The DNA is subsequently used as a template for amplification with the detection of amplicons using fluorescence-generating probes. This method was shown to detect lower levels of HIV virions than NAT methods. The sensitivity of this technology surpasses the high sensitivity of boosted EIA p24 methods and attains a level of sensitivity equal to or surpassing that of NAT.[17,18]

Monitoring HIV proviral DNA

Even though patients starting HAART regimens often achieve non-detectable HIV-1 viral RNA levels, questions remain as to whether or not the proviral DNA, either integrated or un-integrated, responds to HAART. It is not known if relative changes in proviral DNA levels precede changes of viral RNA levels post HAART introduction. Therefore, one of the current challenges in HIV disease management is to detect the presence of low level proviral DNA in latently-infected CD4 lymphocytes and other reservoirs, especially because these hidden reservoirs can replenish and revive viral infection upon activation.[19-21] Thus, a highly reproducible and accurate assay to quantify proviral DNA would enable a more in-depth evaluation of the efficacy of antiviral therapies. Unfortunately, there are no commercially available assays for quantification of HIV-1 proviral DNA levels.

Several research-based assays have been reported previously for quantification of HIV-1 proviral DNA, all of which were based on the principle of conventional PCR.[22-25] The potential limitation associated with the traditional quantitative PCR is that the DNA copy numbers are calculated based on the final amplified gene products. Because DNA is amplified exponentially during PCR, a small variation in amplification efficiency early in the thermocycling process could potentially lead to a large variation in the final quantification of amplified products. To circumvent this problem, we have developed and validated a new protocol for quantification of HIV-1 proviral DNA.[26] This assay is based on the principle of real-time PCR, also known as TaqMan™.[25] Because of the inherent advantage of real-time PCR over classic PCR, this new assay provides a highly accurate and reproducible detection method for HIV-1 proviral DNA with a broad linear range of detection.[26]

DETECTION OF HIV-1 DRUG RESISTANCE

Drug resistance testing by molecular methods now provides valuable information to guide therapy in HIV infected persons. As more information becomes available on the specific mutations that occur during drug treatment, healthcare workers are able to tailor therapy to be more effective in controlling infection and even predicting which drugs will be most efficacious. It is likely that drug resistance testing will gain even wider use as more mutations are detected and as new drugs become available. It is clear that identification of specific mutations provides information that can be translated to a more effective therapy that can extend an infected person's life.

In spite of the striking success of HAART in treating HIV-infected patients, most patients will eventually fail treatment as genetic changes emerge in the virus leading to drug resistance. The observed resistance is usually restricted to changes in those viral genes that serve as targets for the therapies that the patient has begun. For example, patients taking AZT as part of their therapy will typically develop mutations of codon 215 of the reverse transcriptase region of the HIV *pol* gene, thereby leading to a change in a single amino acid of the gene product and decreasing the effectiveness of AZT in the patient. Over time, additional mutations may occur in codons 41 and 219, again leading to different amino acid changes in the reverse transcriptase enzyme. The cumulative acquisition of new mutations is likely to lead to a loss of effectiveness of AZT.[28,29] The identification of mutations can be accomplished through genotyping and phenotyping methods that provide information on the virus's drug resistance or susceptibility. Viral resistance is a decrease in virus susceptibility to a drug as evidenced by an increase in the concentration of drug required to inhibit viral replication by 50%.

The viral genotype reflects the genetic composition of the organism, which generates the phenotypic properties. Genotyping is a method for determining the constitution of nucleotide sequence in the viral genome that encodes the amino acids, and is usually carried out by directly sequencing the DNA of the viral genes. Genotyping assays are used more commonly than the phenotypic assays because they are less expensive, rapid, and more accessible than the phenotypic assays. In addition, it may be able to detect some of the viral gene mutations before drug resistance manifests at the phenotypic level. One of the technical challenges of the genotyping assay is that the knowledge and experience of the analyst may influence the interpretation of results. Moreover, results generated from genotypic assays are not always consistent with the results derived from the phenotypic assays.

The viral phenotype refers to any characteristic of the virus that can be detected or observed and that is related to its appearance, structure, or some measurable property. Phenotypic assays are able to assess the total overall effect of one or multiple mutations and mutational interactions. They evaluate the concentration of different drugs necessary to inhibit HIV replication in vitro, and directly measure, under controlled laboratory conditions, the level of resistance of the HIV population in an individual patient to each of the anti-HIV drugs. The phenotypic assay involves insertion of the reverse transcriptase and protease genes from the HIV clinical isolate of the infected individual via recombinant DNA techniques into a laboratory-derived molecular clone containing standardized viral envelope and accessory genes (experimental test). The recombinant clones are then grown in viral culture in the presence of varying concentrations of the drug being evaluated, enabling an assessment of the phenotypic characteristics expressed by the inserted genes. In addition, a wild-type virus lacking the recombinant inserts is included as a control.

The drug concentrations that result in 50%, 90%, and 95% inhibition of viral growth (in the experimental test) are termed IC_{50}, IC_{90}, or IC_{95}, respectively. Resistance is measured in terms of the IC_{50} and then compared with the IC_{50} for the fully sensitive, non-mutated wild-type virus. A 4-fold or higher shift between the IC_{50} of the recombinant clone and the wild-type virus indicates drug resistance. If the wild-type virus requires 0.5 μg of a drug to reduce viral growth by 50% and the sampled virus required 5 μg, this measurement would be referred to as a 10-fold resistance. Disadvantages of phenotypic assays are their high cost and a long turn around time for reporting (two to three weeks). In addition, the thresholds to define susceptibility are arbitrary, not standardized, and do not always reflect achievable drug concentrations.

The use of automated DNA cycle-sequencing based-technology is currently the most common way to conduct HIV-1 genotyping analysis. Currently, several types of genotypic and phenotypic assays exist for the analysis of HIV drug resistance. The genotyping methods include the FDA-approved TruGene™ HIV-1 Genotyping Kit and Open Gene DNA Sequencing Assay (Visible Genetics/Bayer Diagnostics), and the ViroSeq™ HIV-1 Genotyping System with the 3700 Genetic Analyzer (Celera Diagnostics). Both the ViroSeq HIV-1 Genotyping Systems and the Trugene HIV-1 test offer an integrated software algorithm to provide information to the physician about which gene mutations of RT and PR are

most likely to confer resistance to a specific drug regimen. Inter-laboratory comparison of various DNA sequencing-based genotypic methods indicates that there is a high concordance in determining RT and PR gene mutations.[30]

Currently, the two most popular phenotyping assays are the PhenoSense (ViroLogic, Inc., and the VircoTYPE HIV-1 (Virco). ViroLogic had developed a Therapy Guidance System (TGS) interactive database to help physicians guide patient therapy. The company has also introduced the PhenoSense GT™ system, which combines the PhenoSense HIV phenotyping assay, the GeneSeq HIV genotyping assay, replication capacity (RC), and HIV-1 subtyping to create a comprehensive HIV drug-resistance testing system. RC measures how well a patient's virus is able to replicate compared with a wild-type reference virus. The VircoTYPE HIV-1 testing service was introduced in July 2004 as a complete HIV-1 phenotyping and genotyping service to replace VirtualPhenotype. The VirtualPhenotype (Virco) is a computer software program that provides a phenotypic prediction based on a correlative database of more than 100,000 HIV phenotypes and genotypes (>18,000 paired genotypes and phenotypes).

Another method, the LiPA™ assay (Innogenetics) is a reverse hybridization method that uses easy to read strips for determination of specific viral gene codon changes that confer drug resistance; however, the test only identifies the gene mutations that have been targeted by certain oligonucleotides applied on the strip. Another method that uses the DNA repair enzyme Cleavase™ in the Invader™ assay (Third Wave) could potentially detect all of viral gene mutations, but it is unable to provide specific information on gene codon changes.

One of the most powerful genotypic ways to determine HIV-1 drug resistance is the use of microarray technology. Affimatrix Inc. has developed specific DNA chips that can be used to detect all of the possible gene mutations conferring drug resistance to either RT or PR inhibitors. However, this assay is limited by its high cost of chips and instrument setup.

CONCLUSIONS

The management of HIV infection has changed dramatically in the past several years because of advances in technology that provide more capability for the monitoring of infection and in finding the causes of viral resistance to drug regimens. These capabilities are essential for guiding therapy and to increase the quality and extension of life in those infected with HIV. In light of recent developments in new and improved therapies, we have entered a new era in HIV treatment and management. Nevertheless, drug resistance will continue and vigilance is necessary. Although we by no means have found a cure for HIV infection, we are making good progress and giving more hope for millions of persons infected throughout the world. As we strive to achieve full eradication of HIV infection, we must combine proper therapeutic designs with accurate viral load measurements and resistance testing to allow an assessment of our progress and for the maximum care of patients.

REFERENCES

1. Busch M. Abstract presented at HIV Diagnostics: new developments and challenges, CDC, Orlando, FL, March 2005.
2. Constantine NT, Callahan JD, Watts DM. In: Retroviral testing: essentials for quality control and laboratory diagnosis. CRC Publishers, 1992:235.
3. Constantine NT, Zink H. HIV testing technologies after 2 decades of evolution, Indian J Med Res 2005; 121:519-38.
4. Mellors JW, Rinaldo CR, Gupta P, and others. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Sci 1996;272:1167-70.
5. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 infection. N Engl J Med 1989;132:1621-5.
6. Bartlett, JG, Gallant, JE. Medical management of HIV infection. Baltimore: Johns Hopkins University; 2003.
7. Marschner IC, Collier AC, Cooms RW, and others. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J Infect Dis 1998;177:40-7.
8. Albrecht H, Hoffmann C, Degen O, and others. Highly active antiretroviral therapy significantly improves the prognosis of patients with HIV-associated progressive multifocal leukoencephalopathy. AIDS 1998;12:1149-54.
9. de Mendoza C, Soriano V, Perez-Olmeda M, and others. Different outcomes in patients achieving complete or partial viral load suppression on antiretroviral therapy. J Hum Virol 1999;2:344-9.
10. Guatelli JC, Whitfield KM, Kwoh DY, and others. Isothermal in vitro amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication. Proc Natl Acad Sci USA 1990;87:1874-8.
11. Urdea MS, Horn T, Fultz TJ, and others. Branched DNA amplification multimers for the sensitive, direct detection of human hepatitis viruses. Nucleic Acids Symp Ser 1991;24:197-200.
12. Shingadia D, Zhao Y. Measurement of plasma viral RNA load of human immunodeficiency virus type 1 (HIV-1). Am Med Lab Int 1997;126:4-5.
13. Brambilla D, Reichelderfer PS, Bremmer JW, and others. The contribution of assay variation and biological variation to the total variability of plasma HIV-1 RNA measurements. AIDS 1999;13:2269-79.
14. Ginocchio CC. HIV-1 viral load testing: methods and clinical applications. Lab Med 2001;32:42-52.
15. Yeghiazarian T, Zhao Y, Read SE, and others. Quantification of human immunodeficiency virus type 1 RNA levels in plasma by using small-volume-format branched-DNA assays. J Clin Microbiol 1998;36:2096-8.

16. Barletta JM, Edelman DC, Constantine NT. Lowering the detection limits of HIV-1 viral load using real-time immuno-PCR for HIV-1 p24 antigen. Am J Clin Path 2004;122:20-7.
17. Schupbach J, Boni J, Flepp M, and others. Antiretroviral treatment monitoring with an improved HIV-1 p24 antigen test: an inexpensive alternative to tests for viral RNA. J Med Viro 2001;65:225-32.
18. Schupbach J. Measurement of HIV-1 p24 antigen by signal-amplification-boosted ELISA of heat-denatured plasma is a simple and inexpensive alternative to tests for viral RNA. AIDS Rev 2002;4:83-92.
19. Chun, TW, Stuyver L, Mizell SB, and others. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA 1997;94:13193-7.
20. Finzi D, Hermankova M, Pierson T, and others. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Sci 1997;278:1295-300.
21. Finzi D, Blankson J, Siliciano JD, and others. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med 1999;5:512-7.
22. Bennett JM, Kaye S, Berry N, Tedder RS. A quantitative PCR method for the assay of HIV-1 provirus load in peripheral blood mononuclear cells. J Virol Methods 1991;83:11-20.
23. Christopherson C, Kidane Y, Conway B, and others. PCR-based assay to quantify human immunodeficiency virus type 1 DNA in peripheral blood mononuclear cells. J Clin Microbiol 2000;38:630-4.
24. Guenthner PC, Hart CE. Quantitative, competitive PCR assay for HIV-1 using a microplate-based detection system. Biotechniques 1998;24:810-6.
25. Izopet J, Tamalet C, Pasquier C, and others. Quantification of HIV-1 proviral DNA by a standardized colorimetric PCR-based assay. J Med Virol 1998;54:54-9.
26. Zhao Y, Yu M, Miller JW, and others. Quantification of human immunodeficiency virus type 1 proviral DNA by using TaqMan technology. J Clin Microbiol 2002;40:675-8.
27. Holland P, Abramso R, Watson R, Gelfand D. Detection of specific polymerase chain reaction product by utilizing the 5' - 3' exonuclease activity of *Thermus aquaticus* DNA polymerase. Proc Natl Acad Sci USA 1991;88:7276-80.
28. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Sci 1989;243:1731-4.
29. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Sci 1989;246;1155-8.
30. Demeter LM, D'Aquila R, Weislow O, and others. Interlaboratory concordance of DNA sequence analysis to detect reverse transcriptase mutations in HIV-1 proviral DNA. ACTG Sequencing Working Group. AIDS Clinical Trials Group. J Virol Methods 1998;75:93-104.

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FOCUS: GENE-BASED DIAGNOSTICS

Molecular Diagnostics of Inherited Thrombosis

STACY LEAGUE, W CRAIG HOOPER

Thrombophilia can best be defined as a disorder of coagulation that contributes to a predisposition towards thrombosis. Although the term thrombophilia has been used to describe arterial thrombosis, its most common usage has been in reference to venous thromboembolism (VTE). Thrombophilia can be a consequence of both acquired and inherited or genetic causes. Acquired causes include conditions such as surgery, cancer, and prolonged immobilization, while genetic causes have been linked to the inherited deficiencies of antithrombin, protein C, and protein S. The identification of the genetic basis of these inherited causes of thrombophilia ushered in a new way of thinking about thrombosis and the importance of its genetic component. Interest in the genetic basis of VTE was accelerated with the subsequent discovery of factor V Leiden, prothrombin G20210A, and MTHFR C677T. These single nucleotide polymorphisms (SNPs) and other genetic variants associated with VTE have become fixtures in the molecular diagnosis of inherited thrombophilia. Because of the large volume of current and anticipated future genetic testing, there has been a push to develop many different genotyping methods which are now used in both clinical and research settings. The identification of new genetic variants that may either directly or indirectly affect coagulation or the anticoagulant pathway, may greatly advance the understanding and clinical management of thrombophilia.

ABBREVIATIONS: APCR = activated protein C resistance; CBS = cystathionine β-synthase; FVL = factor V Leiden; LDL = low density lipoprotein; MTHFR = methylenetetrahydrofolate reductase; MTR = methionine synthase; MTRR = methionine synthase reductase; PCR = polymerase chain reaction; PE = pulmonary embolism; PT = prothrombin; SNP = single nucleotide polymorphism; VTE = venous thromboembolism.

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INDEX TERMS: inherited thrombophilia; pulmonary embolism; thrombophilia; thrombosis; venous thromboembolism.

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Focus Continuing Education Credit: *see pages 280 to 283 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES

1. Define thrombophilia and explain the difference between acquired and inherited forms.
2. Define the terms multigenic and multifactorial.
3. Identify the three most common inherited protein deficiencies associated with venous thrombosis.
4. List three common genetic single nucleotide polymorphisms (SNPs) tested in the diagnosis of inherited thrombosis.
5. Describe the mechanism by which the Factor V Leiden mutation affects hemostasis.
6. Describe the effect of the MTHFR C677T mutation on homocysteine metabolism.
7. Explain the purpose of polymerase chain reaction (PCR).
8. Compare and contrast laboratory methods used to identify single nucleotide polymorphisms.

Hemostasis is a complex balance of procoagulant and anticoagulant forces that act in concert to physiologically control

bleeding and clotting. The term thrombophilia refers to a disorder in coagulation that shifts hemostasis towards an increased predisposition for thrombosis. Although thrombophilia has been used by some to describe arterial thrombosis, its usage has commonly been synonymous with VTE and both will be used interchangeably in this review.

With an approximate annual incidence of 1 in 1000, venous thromboembolism (VTE) affects approximately 250,000 individuals each year.[1,2] Pulmonary embolism (PE), a complication of VTE, has a high morbidity rate and is a leading cause of hospital in-patient deaths.[3] Because thrombophilia is both multigenic (involving many different genes) and multifactorial (involving the interaction of genetic and acquired factors) it can be difficult to identify causes and predict risk.

Thrombophilia can be divided into two basic categories: acquired and inherited (Table 1). As implied, in acquired thrombophilia the increased risk of thrombosis is significantly reduced or removed when the associated condition no longer exists, such as the discontinuation of hormone replacement therapy.[4] Inherited thrombophilia is a much more intricate puzzle because of the involvement of a wide array of genes and their proteins that either directly or indirectly affect coagulation and the anti-coagulant pathway. Consequently the possibilities for finding genetic variation related to thrombosis are seemingly endless. In most cases, a genetic predisposition alone is not enough to cause thrombosis. Therefore, the assessment of gene-gene and gene-environment interactions is another important piece of the puzzle. Also adding to the challenge are the differences between ethnicities. For example, the factor V Leiden polymorphism, which will be discussed later, is fairly common in people of Western European descent but found only rarely in those of African or Asian descent.

Table 1. Conditions associated with thrombophilia

Acquired	Inherited
Surgery	Protein C deficiency
Trauma	Protein S deficiency
Malignancy	Antithrombin deficiency
Pregnancy	Dysfibrinogenemia
Oral contraceptives	Factor V Leiden
Hormone replacement therapy	Prothrombin G20210A
Immobilization	
Aging	
Antiphospholipid syndromes	
Hyperhomocysteinemia	
Liver disease	
Sepsis	

GENETIC VARIANTS IMPORTANT IN VTE:

An inherited predisposition can now be identified in approximately 60% to 70% of VTE patients.[5] Autosomal dominant inherited protein C, protein S, and antithrombin deficiencies and, to a much lesser extent, recessive deficiencies or abnormalities in proteins such as factor VII, fibrinogen, and thrombomodulin are thought to account for anywhere from 5% to 15% of venous thromboembolism.[6,7] Over 160 genetic variations have been identified in the protein C gene. In the absence of normal levels and/or decreased functionality of the above-mentioned proteins, the function of other coagulation factors may be impaired. For example, without activated protein C, the procoagulant factors Va and VIIIa are not completely inactivated. Similarly, a deficiency of protein S can impair the function of activated protein C.

Currently, laboratory diagnosis of these deficiencies involves functional or immunological testing, as clinical genetic testing presents somewhat of a challenge. The genetic changes underlying these protein deficiencies are due to many different mutations and types of mutations spread throughout the individual genes, thus making rapid detection difficult. Missense, nonsense, frameshift, deletion, and insertion mutations have all been found (Table 2). Mutation databases for the protein C, protein S, antithrombin, and fibrinogen

Table 2. Types of mutations

Type	Description
Missense	A base change that results in an amino acid substitution.
Nonsense	A base change that results in a stop codon, prematurely terminating the protein.
Insertion	The addition of 1 or more base pairs.
Deletion	The deletion of 1 or more base pairs.
Frameshift	The insertion or deletion that is not a multiple of three within the coding sequence that results in a subsequent change to all the following amino acids.

genes have reported a total of 161, 131, 127, and 282 unique genetic changes, respectively.[8-11] The detection of so many different DNA changes throughout the entire gene(s) is an expensive, time consuming, and labor intensive process. However, since only a single base pair substitution is involved, single nucleotide polymorphisms (SNPs) are ideally suited for molecular testing. The list of SNPs associated with VTE is continually growing (Table 3), but we will focus on three that are among the most commonly tested clinically in thrombophilia: factor V Leiden, prothrombin G20210A, and MTHFR C677T. Other tests clinically available include FXIII Val34Leu, Protein S Heerlen, PAI-1 4G/5G, MTHFR 1298, and platelet GP IIIa Leu33Pro.

FACTOR V LEIDEN

The discovery of activated protein C resistance (APCR) by Dahlbäck in 1993, and the subsequent discovery of the Factor V Leiden (FVL) mutation, which causes at least 90% of

Table 3. Clinical associations of genetic polymorphisms (Modified from Hooper and De Staercke 2002)

Polymorphism	Phenotype	Association with VTE
Protein C anticoagulant pathway		
Factor V Leiden: G1691A (Arg506Gln)	APC resistance	Clear risk factor
Factor V Cambridge: Arg306Thr	APC resistance	Tentative
Factor V Hong Kong: Arg306Gly	APC resistance	Tentative
Factor V HR2 haplotype	Mild APC resistance	Tentative
Thrombomodulin C1481T (Ala455Val)	Unknown	Unlikely
Thrombomodulin-33 G to A	Unknown	Possible
EPCR 23bp insertion in exon 3	Unknown	Unlikely
Protein S Heerlen	Decreased free protein S	Unknown
Procoagulant Proteins		
Prothrombin G20210A	Increased FII	Clear risk factor
Fibrinogen Bcl-1 allele in β chain	Increased fibrinogen	Possible
Fibrinogen-148 CtoT in β promoter	No increased fibrinogen	Not known
Fibrinogen G448A in β chain	Increased fibrinogen	Not known
Fibrinogen Thr312Ala in α chain	Abnormal FXIII cross-linking?	Possible
Factor VII Arg353Gln	Low-normal FVII	Not known
Factor VII H7H7	Low-normal FVII	Not known
Factor VII G73A	Low-normal FVII	Not known
Factor XIII A subunit Val34Leu	Increased activity	Protective?
Homocysteine metabolism		
Cystathionine β-synthase T833C		Possible
5,10-methylenetetrahydrofolate reductase C677T		Unlikely
Platelet surface glycoproteins		
GP IIIa Leu33Pro (PL A2 or HPA-1b)	Increased sensitivity to platelet activation; altered sensitivity to aspirin	Unlikely
GP Iba VNTR	Unknown	Not known
GP Iba C3550T (Thr145Met)	Unknown	Not known
GP Ia/IIa, a2 A1648G	Altered surface expression of receptor	Not known
Thrombin receptor PAR-1-5061D	Unknown	Protective?

APCR, by several groups the following year, revolutionized the molecular diagnosis of thrombophilia.[12-16] The FVL mutation is a G to A transition at position 1691 in the factor V gene which changes arginine 506 to glutamine. Arginine 506 is a cleavage site for activated protein C (Figure 1). When glutamine is present, activated protein C cannot completely inactivate FVa, causing activated protein C resistance. Present in 15% to 20% of patients with VTE and in 2% to 5% of the general U.S. population, it is by far the most common genetic factor in inherited thrombophilia.[17] However, while FVL is common in Caucasian populations, especially those of Western European descent, it is not common in African American and Asian populations.[18,19] Heterozygosity increases the risk of VTE by three- to eight-fold and homozygosity by 50- to 100-fold.[20-22]

Figure 1. Inactivation of Factor Va by activated protein C (APC)

1A: 1) APC cleaves factor Va at Arginine 506; 2) APC cleaves factor Va at Arginine 306; 3) Factor Va is now inactivated.

Factor Va
APC
R306
R506
APC
R306
Inactivated Factor Va

1B: 1) APC cannot cleave factor Va at Glutamine 506; 2) Factor Va remains functional

Factor Va
APC
1
R306
Q506 (Leiden Mutation)
APC
Factor Va
2
R306
Q506 (Leiden Mutation)
Factor Va Still Functional

PROTHROMBIN G20210A

A few years after FVL was reported in 1996, Poort described another single base change associated with VTE, this time in the 3' untranslated region of the prothrombin gene.[23] The G to A transition at position 20210 results in increased levels of circulating prothrombin and hence an increased potential for thrombin generation. As with FVL, PT G20210A is not common in African American or Asian populations, but is found in the Caucasian population at a frequency of 1% to 4%.[24] Among patients with VTE, the frequency rises to 6% to 18% and confers a two- to five-fold increased risk.[23,25-27] Diagnosis of PT G20210A can only be done using molecular genetic testing.

HYPERHOMOCYSTEINEMIA AND MTHFR C677T

In some individuals, plasma levels of the amino acid homocysteine are abnormally high. Hyperhomocysteinemia has been found to increase thrombotic risk by about two- to four-fold.[28] It is believed that atherogenic damage occurs when homocysteine binds to LDL and induces the expression of inflammatory chemokines, such as MCP-1 and IL-8. These chemokines

attract monocytes and neutrophils which attach to endothelial cells, become macrophages, and phagocytose the homocysteine-LDL aggregates. This process forms foam cells, a precursor to atherosclerotic lesion formation.[29] Because there is almost no free homocysteine in foods, circulating plasma homocysteine results from the metabolism of methionine. The enzymes cystathionine, β-synthase (CBS), methionine synthase (MTR), methionine synthase reductase (MTRR), 5,10-methylenetetrahydrofolate reductase (MTHFR), and thymidylate synthase (TS) and several cofactors, betaine, folate, and vitamins B2, B6, and B12, are key components of methionine metabolism. While there are some acquired causes of these elevated levels, such as vitamin B6 or B12 deficiency and renal failure, genetic factors have also been identified. More than 200 different genetic variants have been reported and linked to elevated homocysteine levels.[29] Most are rare and detrimental only when found in the homozygous state or have been found to have no association with VTE. One exception is MTHFR 677 C→T. The mutation results in the substitution of valine for alanine causing a reduction in enzyme activity and an increase in sensitivity to heat inactivation (thermolability).[30,31] A fairly common polymorphism in the general population, MTHFR 677 C→T has been associated with mild hyperhomocysteinemia and considered by some to be a weak VTE risk factor.[32]

GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS

Due to the multigenic and multifactorial nature of VTE, it is possible that two or more genetic changes, or one or more genetic changes and an environmental risk factor, can be present in any individual at any one time. These interactions can have a dramatic effect on risk and consequently it is important to take these interactions into consideration. For instance, patients who are protein C, protein S, or antithrombin deficient and heterozygous for FVL have increased risk of VTE over that of the deficiency alone.[33-36] Significant increases in risk have also been found in patients who carry both the FVL and PT G20210A variants as compared to those with only a single variant.[37] Gene-environment interactions have also been noted between FVL and PT G20210A and oral contraceptives, hormone replacement therapy, pregnancy, and antiphospholipid syndrome.[38-42]

Gene-gene and gene-environment interactions are possible explanations for the conflicting data surrounding the relationship between the MTHFR C677T polymorphism and VTE. While Jacques found elevated plasma homocysteine levels only in individuals who had the T/T genotype and low folate, findings from other studies that looked at interactions were mixed.[43-45]

APPROACHES FOR MOLECULAR ANALYSIS

As mentioned previously, molecular testing for protein C, protein S, antithrombin, and fibrinogen deficiencies requires mutation detection methodology. Genotyping of SNPs such as FVL, PT G20210A, and MTHFR C677T, however, can be done using a wide variety of assays in single or multiplex formats using standard laboratory equipment or specialized instrumentation. Additionally, analyte specific reagents (ASRs) or FDA approved kits are available for some of the polymorphisms. Although they are generally more expensive, the validation and quality control they receive from the manufacturer may justify the extra expense in certain settings. Each assay's accuracy, costs, labor intensity, and multiplexing capabilities should be assessed before deciding on a method to use for a particular study or implement in a clinical laboratory. While it is beyond the scope of this paper to cover all possible methods, several will be discussed and compared.

The polymerase chain reaction (PCR) is the basis of the majority of genotyping methods. PCR allows short fragments of a relatively small amount of genomic DNA to be amplified into quantities suitable for many different types of analysis in only a few hours. Typically, the target DNA is amplified about a million-fold. DNA extracted from tissue, buccal cells, whole blood of certain patients with low white cell counts, or small volumes of blood can have lower DNA concentrations. PCR is especially useful when testing these types of samples. One common PCR-based assay is restriction fragment length polymorphism (RFLP). Although RFLP has not been around for a long time, it is a proven method. While it is quite labor intensive and perhaps not ideally suited for large sample sizes, it is fairly easy to optimize, reagent costs are low, and standard laboratory equipment is all that is necessary to perform the assay. By fluorescently labeling the fragments, throughput can be increased, multiplexing is made easier, and labor intensity decreased with only a minor raise in reagent costs.[46] The major drawback would be the need to purchase a capillary electrophoresis instrument, e.g., ABI Prism® 3100, or Beckman Coulter CEQ™ 8000. Another popular PCR-based method is allele specific amplification (ASA). In this method, each allele is amplified individually and separated by agarose gel or capillary electrophoresis. Although the optimization and design of ASA assays can be more difficult than RFLP, sample manipulation is decreased and therefore less labor is involved.[47,48]

Gene sequencing is generally cost-prohibitive for SNP genotyping. However, Pyrosequencing™ provides 50 to 100 base pairs of sequence data in a cost-effective platform with

assays for FVL and MTHFR C677T already available.[49,50] While there are benefits to having actual sequence data, such as detecting assay problems or nearby polymorphisms, this method requires specialized instrumentation and several post-PCR manipulations, making it fairly labor intensive.

Many new types of assays for SNP genotyping resulted from the advent of fluorescently labeled probe technologies. If carefully optimized, they afford great flexibility because they can be customized to any target sequence and with different fluorophores. Although each type of probe uses a slightly different mechanism to detect the genetic change, they share a common methodology of measuring fluorescent signals after laser excitation. Specific instrumentation is required to perform these assays, but there are many different instruments available in all price ranges, e.g., ABI Prism® 7900, Roche LightCycler®, Stratagene Mx4000®, and Rotor-Gene 2000™. Even though reagent and instrument costs are higher than some other assays, they hold one very distinct advantage: the PCR and genotyping are done simultaneously in the same tube. This significantly decreases manual manipulations, lowering the labor intensity, improving throughput, and decreasing the chance of sample mix-up. Two commonly used assays for genotyping thrombophilic mutations are the Roche Diagnostics LightCycler® Factor V Leiden Mutation and Prothrombin Mutation Detection Kits and Applied Biosystems' TaqMan Allelic Discrimination Assays.[51-55] The LightCycler® has a smaller capacity compared to ABI Prism® 7900 (32 versus 96 wells), but the run times are 45 minutes and 2 to 3 hours, respectively. One advantage to the Roche system is its ability to discriminate between the mutation of interest and other polymorphisms nearby that are not associated with VTE. As an example, there are three rare polymorphisms, two in the factor V gene and one in the prothrombin gene that can cause genotyping errors. The LightCycler® assays are able to discriminate between the different base changes.[56,57] Besides the LightCycler and TaqMan assays, genotyping can also be done using other types of fluorescent probes, such as molecular beacons and Scorpion probes.[58,59]

For high throughput and multiplexing capabilities, arrays such as Nanogen's NanoChip® Systems or GenomeLab's™ SNPstream® Genotyping System, may be a good solution.[60-63] Array technology is well-suited to large sample sizes and is very accurate and precise once optimized. Additionally, an analyte-specific reagent FVL and PT G20210A multiplex kit has been developed by Nanogen. Mass spectrometry is another robust method capable of high throughput and multiplexing when applied to genotyping. Masscode™ SNP Genotyping Technology from BioServe and MassARRAY from Sequenom are two available options.[64,65] Of course, purchasing the specialized instrumentation for these types of assays is a large investment and specialized training is required.

The Luminex® platform has also been utilized for SNP genotyping, taking advantage of its capacity for multiplexing and high throughput using fluorescently labeled microspheres and flow cytometry.[66,67] While assays can be custom-designed, kits are available from Tm Bioscience to test FVL alone, multiplexed with PT G20210A, or multiplexed with PT G20210A, MTHFR 677, and MTHFR 1298. Again, special instrumentation is necessary and there are several post-PCR manipulations, but it is a flexible platform, not only for genotyping, but for measurement of proteins as well.

Variations of single base extension (SBE) and oligonucleotide ligation assay (OLA) are additional PCR-based choices.[68-71] One non-PCR based option of note is the Invader® assay from Third Wave Technologies.[72,73] This assay requires only a plate reader and heat block, inexpensive items found in

Table 4. Methodology comparison

Method	Labor intensity	Throughput	Cost	Special instrument needed	ASR reagents
RFLP	Med-High	Low-Med	Low-Med	If fluorescently labeled	No
ASA	Med-High	Low-Med	Low-Med	If fluorescently labeled	No
Pyrosequencing	Med	Med	Med	Yes	No
Probe-based	Low	Med	Med	Yes	Yes
Arrays	Med	High	High	Yes	Yes
Mass spectrometry	Med	High	High	Yes	No
Luminex	Med	High	Med	Yes	Yes
Invader	Med	Med	Med	No	Yes

most laboratories, and with an assay set-up similar to that of PCR, technician time is minimal. Several additions and incubations are required before reading the plate. No assay development would be required for FVL, PT 20210, and MTHFR as ASR kits have already been developed.

Obviously, there are many choices for SNP genotyping, including some not mentioned here. As each method has its own advantages and disadvantages in regards to cost, accuracy, and labor intensity, careful evaluation and validation should be performed for each assay under consideration to find the best fit (Table 4). When testing in a clinical setting, using FDA approved or ASR reagents may be worth their additional expense. As always, a comparison of how several methods perform in your own hands is always the best way to make any decisions.

FUTURE DIRECTIONS

Currently, the value of genetic testing is somewhat limited, as the results do not usually change medical treatment and issues regarding privacy, genetic testing, and genetic counseling of unaffected carriers have yet to be resolved.[74] Even though the utility of molecular diagnosis in thrombophilia is somewhat limited at this time, it will surely play a larger role in the future. As gene sequencing continues to become less expensive and labor intensive, candidate gene sequencing of additional proteins involved in hemostasis will continue at a rapid pace, identifying more genetic polymorphisms correlated to an increased risk of VTE. For instance, it has been found that elevated levels of fibrinogen, factor VIII, factor IX, and factor XI are all risk factors for VTE.[75-80] Genetic components for these increases have not yet been identified, but may be in the future.

Another area in which a great deal of research is being done is the effects of inflammation and the immune response on hemostasis. As our comprehension of the roles that they play in thrombosis increases, the molecular diagnosis of thrombophilia will most likely expand to include genes in these pathways and provide new candidates for drug therapies. Genes involved in the metabolism of drugs, such as Cytochrome P450, may also play a central role in the treatment of thrombophilia. Identifying particular genotypes that require higher doses of anticoagulant drugs for appropriate therapeutic effects or genotypes that require lower doses to prevent bleeding complications will be an important step forward in using molecular diagnosis for patient care.[81] Additionally, as the ability to multiplex many SNPs in a cost-effective way using small amounts of sample continues to improve, the understanding of gene-gene interactions will leap forward. A patient's genetic information will be an important piece in solving the puzzle of thrombophilia.

REFERENCES

1. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med 2001;344:1222-31.
2. Silverstein MD, Heit JA, Mohr DN, and others. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med 1998;158:585-93.
3. Kearon C, Salzman EW, Hirsh J. Epidemiology, pathogenesis, and natural history of venous thrombosis: In Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, editors. Hemostasis and thrombosis. 4th ed. Baltimore: Lippincott, Williams & Wilkins. 2001. p 1153-77.
4. Gomes MPV, Deitcher SR. Risk of venous thromboembolic disease associated with hormonal contraceptives and hormone replacement therapy. Arch Intern Med 2004;164:1965-76.
5. Buchanan GS, Rodgers GM, Branch DW. The inherited thrombophilias: genetics, epidemiology, and laboratory evaluation. Best Prac Res Clin Obstet Gynaec 2003;17:397-411.
6. Franco RF, Reitsma PH. Genetic risk factors of venous thrombosis. Hum Genet 2001;109:369-84.
7. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. Blood 1996;87:3531-44.
8. Mariani G, Herrmann FH, Schulman S, and others. Thrombosis in inherited factor VII deficiency. J Thromb Haemost 2003;1:3-9.
9. Protein C database: www.xsrall.nl/~reitsma/Prot_C_home.htm. Accessed May 25, 2005.
10. Protein S database: www.med.unc.edu/isth/proteins.htm. Accessed May 25, 2005.
11. Antithrombin database: www.med.ic.ac.uk/divisions/7/antithrombin/index.htm. Accessed May 25, 2005.
12. Fibrinogen database: www.geht.org/pages/database_ang.html. Accessed May 25, 2005.
13. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993;90:1004-8.
14. Bertina RM, Koeleman RPC, Koster T, and others. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
15. Greengard JS, Sun X, and others. Activated protein C resistance caused by Arg506Gln mutation in factor Va. Lancet 1994;343:1362-3.
16. Voorberg J, Roelse J, Koopman R, and others. Association of idiopathic thromboembolism with single point mutation at Arg506 of factor V. Lancet 1994;343:1535-6.
17. Zoller B, Dahlback B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. Lancet 1994;343:1536-8.
18. Hooper WC, Evatt BL. The role of protein C resistance in the pathogenesis of venous thrombosis. Am J Med 1998;316:120-8.
19. Gregg JP, Yamane AJ, Grody WW. Prevalence of the factor V-Leiden mutation in four distinct American ethnic populations. Am J Med Genet 1997;73:334-6.
20. Pepe G, Rickards O, Vanegas OC, and others. Prevalence of factor V Leiden mutation in non-European populations. Thromb Haemost 1997;77:329-31.

21. Koster T, Rosendaal FR, Ronde H, and others. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 1993;342:1503-6.
22. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994;330:517-22.
23. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995;85:1505-8.
24. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-703.
25. Rosendaal FR, Doggen CJ, Zevelin A, and others. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79:706-8.
26. Cumming AM, Keeney S, Salden A, and others. The prothrombin gene G20210A variant: prevalence in a UK anticoagulant clinic population. Br J Haematol 1997;98:353-5.
27. Kapur RK, Mills LA, Spitzer SG, Hultin MB. A prothrombin gene mutation is significantly associated with venous thrombosis. Arterioscler Thromb Vasc Biol 1997;17:2875-9.
28. Rosendaal FR, Doggen CJ, Zivelin A and others. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79:706-8.
29. Falcon CR, Cattaneo M, Panzeri D, and others. High prevalence of hyperhomocysteinemia in patients with juvenile venous thrombosis. Arterioscler Thromb 1994;14:1080-3.
30. McCully KS. Homocysteine, vitamins, and prevention of vascular disease. Mil Med 2004;4:325-9.
31. Kang SS, Zhou J, Wong PWK, and others. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet 1988;43:414-21.
32. Frosst P, Blom HJ, Milos R, and others. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genet 1995;10:111-3.
33. Ray JG, Shmorgun D, Chan WS. Common C677T polymorphism of the methylenetetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies. Pathophysiol Haemost Thromb 2002;32:51-8.
34. Hallam PJ, Millar DS, Krawkzac M, and others. Population differences in the frequency of the factor V Leiden variant among people with clinically symptomatic protein C deficiency. J Med Genet 1995;32:543-5.
35. Zöller B, Berntsdotter A, Garcia de Frutos P, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in inherited deficiency of protein S. Blood 1995;35:3518-23.
36. Boven HH, Reitsma PH, Rosendaal FR, and others. Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. Thromb Haemost 1996;75:417-22.
37. Salomon O, Steinberg DM, Zivelin A, and others. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism. Prevalence and risk assessment. Arterioscler Thromb Vasc Biol 1999;19:511-8.
38. Vandenbroucke JP, Koster T, Briet E, and others. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of the factor V Leiden mutation. Lancet 1994;344:1453-7.
39. Martinelli I, Taioli E, Bucciarelli P, and others. Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. Arterioscler Thromb Vasc Biol 1999;19:700-3.
40. Rosendaal FR, Helmerhorst FM, Vendenbroucke JP. Oral contraceptives, hormone replacement therapy, and thrombosis. Thromb Haemost 2001;86:112-23.
41. Dilley A, Austin H, El-Jamil M, and others. Genetic factors associated with thrombosis in pregnancy in a United States population. Am J Obstet Gynecol 2000;183:1271-7.
42. Simantov R, Lo SK, Salmon JE, and others. Factor V Leiden increases the risk of thrombosis in patients with antiphospholipid antibodies. Thromb Res 1996;84:361-5.
43. Jacques PF, Kalmbach R, Bagley PJ, and others. The relationship between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. J Nutr 2002;132:283-8.
44. Gemmati D, Ongaro A, Scapoli GL, and others. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. Cancer Epidemiol Biomarkers Prev 2004;13:787-94.
45. Alessio ACM, Annichino-Bizzacchi JM, and others. Polymorphisms in the methylenetetrahydrofolate reductase and methionine synthase reductase genes and homocysteine levels in Brazilian children. Am J Med Gen 2004;128A:256-60.
46. Benson JM, Ellingsen D, Renshaw MA, and others. Multiplex analysis of mutations in four genes using fluorescence scanning technology. Thromb Res 1999;96:57-64.
47. Kirschbaum NE, Foster PA. The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance. Thromb Haemost 1995;74:874-8.
48. Liu Q, Thorland EC, Heit JA, Sommer SS. Overlapping PCR for bidirectional PCR amplification of specific alleles: a rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes. Genome Res 1997;7:389-98.
49. Ronaghi M, Karamohamed S, Petterson B, and others. Real-time DNA sequencing using detection of pyrophosphatate release. Anal Biochem 1996;242:84-9.
50. Berg LM, Sanders R, Alderborn A. Pyrosequencing technology and the need for versatile solutions in molecular clinical research. Expert Rev Mol Diagn 2002;2:361-9.
51. Happich D, Schwaab R, Hanfland P, Hoernschemeyer D. Allelic discrimination of factor V Leiden using a 5' nuclease assay. Thromb Haemost 1999;82:1294-6.
52. Benson JM, Ellingsen D, Bonduris MK, and others. factor V Leiden and Factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb Haemost 2001;86:1188-92.
53. Lay MJ, Wittwer CT. Real-time fluorescence genotyping of factor V Leiden during rapid-cycling PCR. Clin Chem 1997;43:2262-7.
54. Schroell-Metzger B, Dicato M, Bosseler M, Berchem G. Comparison of standard PCR and the LightCycler technique to determine the thrombophilic mutations: an efficiency and cost study. Clin Chem Lab Med 2003;41:482-5.

55. Nauck M, Marz W, Wieland H. Evaluation of the Roche Diagnostics LightCycler-Factor V Leiden Mutation Detection Kit and the LightCycler-Prothrombin Mutation Detection Kit. Clin Biochem 2000;33:213-6.
56. Lyon E, Millson A, Phan T, Wittwer C. Detection and identification of base alterations within the region of factor V Leiden by fluorescent melting curves. Mol Diagn 1998;3:203-10.
57. Warshawsky I, Hren C, Sercia L, Shadrach B, and others. Detection of a novel point mutation of the prothrombin gene at position 20209. Diagn Mol Pathol 2002;11:152-6.
58. Thelwell N, Millington S, Solinas A, and others. Mode of action and application of Scorpion primers to mutation detection. Nucleic Acids Res 2000;28:3752-61.
59. El Housni H, Heimann P, Parma J, Vassart G. Single-nucleotide polymorphism genotyping by melting analysis of dual-labeled probes: examples using factor V Leiden and prothrombin 20210A mutations. Blood Coagul Fibrin 2003;14:421-4.
60. Schrijver I, Lay MJ, Zehnder JL. Diagnostic single nucleotide polymorphism analysis of factor V Leiden and prothrombin 20210 G>A. A comparison of the Nanogen Electronic Microarray with restriction enzyme digestion and the Roche LightCycler. Am J Clin Pathol 2003;119:490-6.
61. Evans JG, Lee-Tataseo C. Determination of the factor V Leiden single nucleotide polymorphism in a commercial clinical laboratory by use of NanoChip microelectric array technology. Clin Chem 2002;48:1406-11.
62. Erali M, Schmidt B, Lyon E, Wittwer C. Evaluation of electronic microarrays for genotyping factor V, factor II, and MTHFR. Clin Chem 2003;49:732-9.
63. Bell PA, Chaturvedi S, Gelfand CA, and others. SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. Biotechniques 2002;June Suppl:70-2,74,76-7.
64. Kokoris M, Dix K, Moynihan K, and others. High-throughput SNP genotyping with the Masscode system. Mol Diagn 2000;5:329-40.
65. Jurinke C, van den Boom D, Cantor CR, Koster H. The use of MassARRAY technology for high throughput genotyping. Adv Biochem Eng/Biotech 2002;77:57-74.
66. Taylor JD, Briley D, Nguyen Q, and others. Flow cytometric platform for high-throughput single nucleotide polymorphism analysis. Biotechniques 2001;30:661-6, 668-9.
67. Sherrill CB, Marshall DJ, Moser MJ, and others. Nucleic acid analysis using an expanded genetic alphabet to quench fluorescence. J Am Chem Soc 2004;126:4550-6.
68. Chakravarty A, Hansen TS, Horder M, Kristensen SR. A fast and robust dual-label nonradioactive oligonucleotide ligation assay for detection of factor V Leiden. Thromb Haemost 1997;78:1234-6.
69. Benson JM, Phillips DJ, Holloway BP, and others. Oligonucleotide ligation assay for detection of the factor V mutation (Arg506>Gln) causing protein C resistance. Thromb Res 1996;83:87-96.
70. Mas VR, Fisher RA, Maluf DG, and others. Polymorphisms in cytokines and growth factor genes and their association with acute rejection and recurrence of hepatitis C virus disease in liver transplantation. Clin Genet 2004;65:191-201.
71. Hsu TM, Chen X, Duan S, and others. Universal SNP genotyping assays with fluorescence polarization detection. Biotechniques 2001;31:560-70.
72. Hessner MJ, Budish MA, Friedman KD. Genotyping of factor V G1691A (Leiden) without the use of PCR by invasive cleavage of oligonucleotide probes. Clin Chem 2000;46:1051-6.
73. Kwiatkowski RW, Lyamichev V, de Arruda M, Neri B. Clinical, genetic, and pharmacogenetic applications of the Invader assay. Mol Diagn 1999;4:353-64.
74. Grody WW, Griffin JH, Taylor AK, and others. American College of Medical Genetics Consensus Statement on Factor V Leiden Mutation Testing. Genetics Med 2001;3:139-48.
75. Koster T, Blann AD, Briët E, and others. Role of clotting factor VIII and effect of von Willebrand factor on the occurrence of deep-vein thrombosis. Lancet 1995;345:152-5.
76. O'Donnell J, Tuddenham EG, Manning R, and others. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction. Thromb Haemost 1997;77:825-8.
77. Kamphuisen PW, Eikenboom JC, Vos HL, and others. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. Thromb Haemost 1999;81:680-3.
78. Rosendaal FR. High levels of factor VIII and venous thrombosis. Thromb Haemost 2000;83:1-2.
79. Meijers JC, Tekelenburg WL, Bouma BN, and others. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 2000;342:696-701.
80. Hylckama Vlieg A van, Linden IK van der, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. Blood 2000;95:3678-82.
81. You JH, Chan FW, Wong RS, Cheng G. The potential clinical and economic outcomes of pharmacogenetics-oriented management of warfarin therapy-a decision analysis. Thromb Haemost 2004;92:590-7.

Continuing Education Questions

FALL 2005

To receive 3.5 contact hours of advanced level P.A.C.E.® credit for the **Focus: Gene-based Diagnostics I** questions, 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 being filled matches the number of the question being answered.

LEARNING OBJECTIVES

1. Describe the classification of gene-based amplification methods.
2. State the principles of gene-based amplification methods.
3. Discuss the advantages and disadvantages of gene-based diagnostics.
4. Define future prospectives of gene-based diagnostics.
5. Describe the uses of molecular-based assays in addressing issues related to HIV infection.
6. List three specific molecular methods commonly used to quantify HIV viral load and describe how they differ in principle.
7. Describe molecular methods that can be used to determine viral resistance to anti-retroviral drugs.
8. Define thrombophilia and explain the difference between acquired and inherited forms.
9. Define the terms multigenic and multifactorial.
10. Identify the three most common inherited protein deficiencies associated with venous thrombosis.
11. List three common genetic single nucleotide polymorphisms (SNPs) tested in the diagnosis of inherited thrombosis.
12. Describe the mechanism by which the factor V Leiden mutation affects hemostasis.
13. Describe the effect of the MTHFR C677T mutation on homocysteine metabolism.
14. Explain the purpose of polymerase chain reaction (PCR).
15. Compare and contrast laboratory methods used to identify single nucleotide polymorphisms.

FROM SINGLE CELL GENE-BASED DIAGNOSTICS TO DIAGNOSTIC GENOMICS: CURRENT APPLICATIONS AND FUTURE PERSPECTIVES

1. Select the gene-based amplification method.
   a. Target-based
   b. Probe-based
   c. Signal-based
   d. All of the above

2. Which technique does **NOT** use a probe-based amplification method?
   a. Real-time PCR
   b. Qβ-replicase
   c. LCR
   d. SDA

3. Which one of the following statements is **NOT** true?
   a. Strand displacement assay (SDA) resembles a DNA repair process.
   b. PCR mimics jellyfish DNA synthesis.
   c. NASBA resembles retroviral replication.
   d. Qβ-replicase assay imitates bacteriophage replication.

4. Which one of the following statements about the conventional PCR is **NOT** correct?
   a. Conventional PCR amplifies DNA in an exponential way.
   b. A small variation in DNA templates could lead to a large variation of the amplified final products.
   c. Conventional PCR normally has a limited linear dynamic range of detection.
   d. Final quantity of the amplicons can be used to determine copy number of the DNA template.

5. Which of the following is a fluorescent label currently used as detection marker in molecular diagnostics?
   a. FAM (SYBR Green I)
   b. JOE (VIC)
   c. TAMRA (NED or Cy3)
   d. ROX (Texas Red)
   e. All of the above

6. Gene-based diagnostic methods have all of the following advantages over serological assays **EXCEPT**:
   a. high sensitivity and specificity.
   b. no need for specimen cultivation.
   c. low error rate.
   d. diagnosis of HIV infection in newborns.

7. Which one of the following assays potentially has the highest sensitivity?
   a. Real-time PCR
   b. LCM
   c. iPCR
   d. BCA

8. What is MALDI-TOF-MS used for?
   a. Protein isolation in proteomic assay
   b. Protein sequence identification
   c. Formation of protein tertiary structure
   d. DNA extraction

9. Which of the following statements closely describes the potential role of quantum dots in gene-based diagnostics? Qdots potentially have the highest:
   a. Amplification power
   b. Specificity power
   c. Detection power
   d. All of the above

10. Which of the following statements closely describes the nature of BCA?
    a. BCA is a target-based amplification assay
    b. BCA is a probe-based amplification assay
    c. BCA is a signal-based amplification assay
    d. None of the above

MOLECULAR-BASED LABORATORY TESTING AND MONITORING FOR HUMAN IMMUNO-DEFICIENCY VIRUS INFECTIONS

11. Which of the following is **NOT** an appropriate use of molecular HIV tests:
    a. Confirming HIV infection in persons who have positive antibody tests
    b. Detection of HIV infection during the serologic window period
    c. Monitoring viral load for prognostic purposes
    d. Monitoring the effectiveness of anti-retroviral therapy

12. How many days can a molecular method detect HIV infection earlier than antibody tests?
    a. 3 days
    b. 5 days
    c. 10 days
    d. 60 days

13. Which of the following molecular methods relies on signal amplification:
    a. RT-PCR
    b. bDNA
    c. NASBA
    d. PCR

14. HIV viral load peaks early during infection to levels of about:
    a. 500 copies/mL
    b. 10,000 copies/mL
    c. 1 million copies/mL
    d. 1 billion copies/mL

15. Most of the molecular assays for HIV have a lower range of detection of:
    a. 50 copies/mL.
    b. 1,000 copies/mL.
    c. 2,000 copies/mL.
    d. 5,000 copies/mL.

16. Which of the following methods does **NOT** rely on isothermal amplification?
    a. NASBA
    b. TMA
    c. RT-PCR
    d. bDNA

17. Which of the following is often used to determine mutations associated with anti-retroviral drugs?
    a. TMA
    b. NASBA
    c. bDNA
    d. Genotyping

18. DNA PCR is usually used to:
    a. detect HIV infection in newborns.
    b. detect viral resistance.
    c. detect DNA in serum samples.
    d. stage HIV infection.

19. In clinical laboratories, amplicons, generated from amplification methods, are usually detected by:
    a. colorimetric or chemiluminescent methods.
    b. gel electrophoresis.
    c. radioisotopes.
    d. Southern blotting.

20. Which of the following methods for determining gene mutations is the most expensive and has the longest turn around time for results?
    a. Genotyping
    b. Phenotyping
    c. Virtual genotyping

MOLECULAR DIAGNOSTICS OF INHERITED THROMBOSIS

21. Thrombophilia is defined as:
    a. a tendency towards bleeding.
    b. a shift in hemostasis towards an increased predisposition for thrombosis.
    c. an equal balance between the prothrombotic and antithrombotic components of hemostasis.
    d. a protein deficiency.

22. A multigenic disease is one that:
    a. has many different symptoms.
    b. is caused by both environmental and genetic factors.
    c. can be caused by genetic variations in more than one gene.
    d. creates new genetic changes in an individual.

23. Which type of thrombophilia is due to environmental factors such as hormone replacement therapy or surgery?
    a. Acquired
    b. Inherited
    c. Neither of the above
    d. Both of the above

24. The three most common inherited protein deficiencies associated with VTE are:
    a. factor VIII, factor IX, and factor VII.
    b. protein C, protein S, and fibrinogen.
    c. cystathionine-β-synthase, methylenetetrahydrofolate reductase, and methionine synthase.
    d. factor V, prothrombin, and factor XIII.

25. FVL, PT G20210A, and MTHFR C677T are:
    a. protein deficiencies associated with VTE.
    b. three SNPs commonly tested in inherited thrombophilia.
    c. methods used in molecular testing.
    d. acquired causes of thrombophilia.

26. The FVL polymorphism causes ___________ because it alters a protein C cleavage site, not allowing factor Va to be inactivated.
    a. hyperhomocysteinemia
    b. activated protein C resistance
    c. acquired thrombophilia
    d. abnormal fibrinogen cross-linking

27. When the MTHFR C677T variant is present, enzyme activity is _________, causing an ___________ in plasma homocysteine levels.
    a. increased; increase
    b. increased; decrease
    c. decreased; increase
    d. decreased; decrease

28. PCR is a molecular method used to:
    a. measure the amount of a protein.
    b. measure the function of a protein.
    c. link small pieces of DNA together into larger fragments.
    d. create many copies of a short fragment of DNA for further analysis.

29. Which one of the following molecular methods would be ideally suited to very large sample sizes where high throughput is needed?
    a. ASA
    b. Probe-based
    c. Luminex
    d. RFLP

Continuing Education Registration Form

To earn continuing education (P.A.C.E.®) credit, (1) complete the form below, (2) record your answers, and (3) tear out and mail this form with a check or money order ($18 for ASCLS members, $28 for non-members for all articles) to:

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Answers
Circle correct answer (questions are on previous three pages).

1. a b c d e	9. a b c d e	17. a b c d e	25. a b c d e
2. a b c d e	10. a b c d e	18. a b c d e	26. a b c d e
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4. a b c d e	12. a b c d e	20. a b c d e	28. a b c d e
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6. a b c d e	14. a b c d e	22. a b c d e	30. a b c d e
7. a b c d e	15. a b c d e	23. a b c d e	31. a b c d e
8. a b c d e	16. a b c d e	24. a b c d e	32. a b c d e

Participant Information
Please circle the most appropriate answers.

1. Is this program used to meet your CE requirements for:
(a) state license (b) NCA (c) employment (d) other

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?

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Abbreviations Used:
AB = Abstract
CP = Clinical Practice
DD = Dialogue and Discussion
FO = Focus
LE = Letter to Editor
RR = Reports and Reviews
RS = Research
TT = Trends and Technology
WB = Washington Beat

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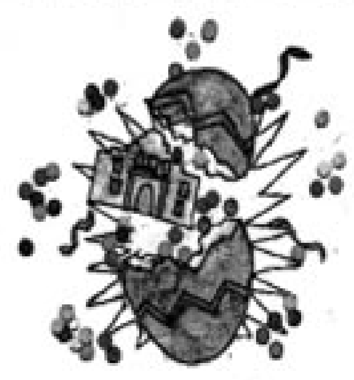

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