**ASCLS Vision Statement**

The American Society for Clinical Laboratory Science, as the pre-eminent organization for clinical laboratory science practitioners, provides dynamic leadership and vigorously promotes all aspects of clinical laboratory science practice, education and management to ensure excellent, accessible, cost-effective laboratory services for the consumers of health care.

**ASCLS Mission Statement**

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

**ADDRESS CHANGES**

Postmaster: Send address changes to Clinical Laboratory Science, 6701 Democracy Blvd, Suite 300, Bethesda MD 20814.

**ASCLS MEMBER EDITORS**

Editor-in-Chief
Susan J Leclaire PhD CLS(NCA)
Department of Medical Laboratory Science
University of Massachusetts Dartmouth
North Dartmouth MA 02747-2715
leclaire@umassd.edu

Continuing Education Editors
Carol McCoy PhD CLS(NCA)
Department of Clinical Science, 4096 HSC
University of Wisconsin–La Crosse
La Crosse WI 54601
mcocar@uwla.edu

Bernadette Redak MS CLS(NCA)
Clinical Laboratory Science
Indiana University, 409 Everser
1120 South Avenue
Indianapolis IN 46202-5133
brodak@iu.edu

Clinical Practice Editor
Isaac D Montoya PhD CMC CLS(NCA)
Affiliated Systems Corporation
3104 Edloc, Suite 330
Houston TX 77027-6022
imontoya@affiliatedsystems.com

P.A.C.E.* Liaison
Sharon Miller/St Charles IL

Contributing Editors
Eileen Carreiro/N Dartmouth MA
George Frisuta/Trusville AL
Sandra Heatherley/Corpus Christi TX
Rebecca Laucidina/Chapel Hill NC
Coonie Mahon/San Antonio TX
Teresa Naldner/Richmond VA
Claudette Ryan/Nashville TN
Linda Smith/San Antonio TX
Michelle Wight-Kanuth/Galveston TX

**REVIEW BOARD**

Richard Bamberg/Greenville NC
Kathleen Blevins/Oklahoma City OK
Dianne Gearlock/DeKalb IL
Peter Colaninno/Jamaica NY
Jo Ann Penn/Salt Lake City UT
Ellis Frohman/St Louis MO
Mildred Fuller/Norfolk VA
Abraham Furman/Portland OR
Richard Gregory/Indianapolis IN
Denise Harmon/Baltimore MD
Linda Hogan/Wichita KS
Jean Holter/Morgantown WV
Cherry Horn/Washington NC
Virginia Hughes/Montgomery AL
Elizabeth Kenimer/Augusta GA
Nancy Konopka/Greensboro PA
Linda Laatsch/Milwaukee WI
Hal Larsen/Lubbock TX
Lou/Ann Lawrence/New Orleans LA
Donna Leach/Winston-Salem NC
Lauralyn Lebeck/La Jolla CA
Craig Lehman/Stony Brook NY
Lynn Littel/Dallas TX
David McGlashon/Lackland AFB TX
Shirlyn McKenzie/San Antonio TX
Sharon Miller/St Charles IL
Harriette Nadler/King of Prussia PA
Allison Pohl/Kalamazoo MI
Jean Prince/Milwaukee WI
Margaret Reinhart/Philadelphia PA
John Seabolt/Lexington KY
Catherine Sheehan/Middletown RI
Stephen Sodeker/Tinquex AL

Advertising for CLS is accepted in accordance with the advertising policy of the ASCLS. Contact the CLS advertising representative at (301) 657-2768.

**CLINICAL LABORATORY SCIENCE**

6701 Democracy Blvd, Suite 300
Bethesda, Maryland 20817
(301) 657-2768, (301) 657-2909 (fax)
www.ascls.org/

**Published quarterly by the American Society for Clinical Laboratory Science, 6701 Democracy Blvd., Suite 300, Bethesda MD 20814; (301) 657-2768; (301) 657-2909 (fax).**

**Annual Subscription Rates:**

- USA
- Canada
- Non-USA

- Individuals $40 $65 $100
- Institutions $60 $65 $100

The cost of single copies is $10. Requests to replace missing issues free of charge are honored up to 6 months after the date of issue. Send requests to ASCLS headquarters. Annual membership dues of ASCLS are $80, $40 of which is allocated to a subscription of CLS. Periodical postage paid at Bethesda, MD and other additional mailing offices.

All articles published represent the opinions of the authors and do not reflect the official policy of ASCLS or the authors’ institutions unless specified.

Microfilm and microfiche editions of CLS are available from University Microfilms, 300 N Zeeb Road, Ann Arbor MI 48106. Correspondence related to editorial content should be mailed to: CLS Editorial Office, 1405 11th Street, PO Box 5399, Coralville IA 52241-5399 (319) 351-2922; (319) 351-2927 (fax)

cbi@ia.net

www.ascls.org/leadership/cls/index.htm

Executive Editor
Marian Schwabbauer PhD

Managing Editor
Ivan Schwabbauer

Trends and Technology Editor
Mary Jane Gore
6701 Democracy Blvd, Suite 300
Bethesda MD 20814
chlstr@aol.com

PRODUCTION
BB Design Studio
2416 E Avenue NE
Cedar Rapids IA 52402

© Copyright 2003 American Society for Clinical Laboratory Science Inc. All rights reserved.
DIALOGUE AND DISCUSSION

66 Editorial: Future Directions for the Clinical Laboratory Scientist
  Carol McCoy

67 Letter to the Editor

68 Washington Beat: CLIA Regulations Updated
  Kathy Hansen, Don Lavanty

CLINICAL PRACTICE

70 Clinical Laboratory Educators Conference 2003 Abstracts

79 Trans Type Genotype Alpha Thalassemia Trait: A Case Study
  Angela B Foley, Louann W Lawrence

82 Career Alternative: Clinical Trials
  Peter M Colaminno

85 HIPAA Privacy Rule: The Debate Continues
  Karrie B Hovis, Dianna M Veillon

ASCLS 2003 ANNUAL MEETING PROGRAM

REPORTS AND REVIEWS

94 Method Comparison Studies for Prostate Specific Antigen and Unconjugated Estriol Immunoassays
  Mary E Koenn, Boniface V Ndah

RESEARCH

99 Validity of Injecting Drug Users’ Self Report of Hepatitis A, B, and C
  Erin G Schlicting, Mark E Johnson, Christiane Brems, Rebecca S Wells, Dennis G Fisher, Grace Reynolds

FOCUS: HEMORRHAGIC ABNORMALITIES

107 Managing the Bleeding Patient
  George A Fritsma

111 Laboratory Management of the Bleeding Patient
  Laura J Taylor

115 Use of Blood Products and Factor Concentrates for Coagulation Therapy
  Margaret G Fritsma

120 Treatment of Single Factor Deficiencies: A Case Study Approach
  Marisa B Marques

CONTINUING EDUCATION QUESTIONS

123

TRENDS AND TECHNOLOGY

127
Future Directions for the Clinical Laboratory Scientist

CAROL MCCOY

Editorials serve many purposes: one is to communicate an issue or concern, another is to provide an overview of the journal articles, and a third is to generate discussion from its readers. The Winter 2003 editorial encouraged readers to become more involved in the profession and perhaps submit articles for publication. The Spring 2003 editorial...well, you can decide its purpose.

Some time ago, I posed the question...“is it time to look at an entry level master’s degree for the clinical laboratory scientist?” Justification ranged from keeping up with the other professions that are moving or have already moved to the master’s level, to the knowledge required to function as a CLS exceeds the baccalaureate degree. CLS educators complain that the body of knowledge of the profession can not be adequately covered in four years. The changing responsibilities for the CLS requires more didactic time to learn laboratory operations, financial management, regulatory compliance issues, clinical correlation, and research design. Thus, an entry level master’s would afford more time to adequately cover the material. This suggestion has not met with unanimous support. Arguments against the idea include low salaries, increased shortages in manpower, and uncertainty of the role the hospital-sponsored programs could play in this type of education program. Even so, NAACLS has appointed a task force to evaluate the move to the graduate level as a future direction for the profession.

Setting emotions aside, look at where the education and responsibilities of the CLS and CLT are in 2003 compared to ten to twenty years ago. The NAACLS Standards (2001) for the CLS/MT and CLT/MLT describe as career entry responsibilities for the CLT/MLT as...“the primary analyst making specimen oriented decisions on pre-determined criteria...”, while the CLS/MT responsibilities go beyond the testing to include clinical decision-making, regulatory compliance, quality assurance/process improvement, evaluation of test systems, all aspects of laboratory management, and adequate knowledge of research design principles to evaluate published studies. Truly the entry-level knowledge required of the CLS has gone beyond the baccalaureate level.

Some programs view the suggestion of entry level master’s as a threat to their continuance. How does a hospital-sponsored program fit into a master’s level program? Several models can be developed to accomplish this task. The main ingredient is collaboration between the hospital program and the university/college.

For the employer, what would be the advantage of the change? In many institutions there is no clear cut distinction between the roles of the CLS versus CLT. Many institutions advertise for a CLS or a CLT. The message communicated is that work functions are not separated by competencies. It appears the employer expectations of a baccalaureate-prepared CLS are less than the capabilities of the graduate. The required competencies should dictate the qualifications requested, not advertising for either in the event the baccalaureate-prepared is not available. The employer would be able to better utilize his/her resources.

One may ask how does this help the shortage of qualified personnel issue? Through better delineation of functions, it will become apparent the CLT can assume much of the analytical testing and free the CLS to perform at a level that allows his/her to better use his/her education. The education of the CLS prepares the graduate to function as a generalist, specialist, educator, or manager. The CLT is prepared to function, with the supervision of the CLS, as the primary analyst in the clinical laboratory.

The Futures Conference held in Chicago identified roles for the CLT and CLS in the next five and ten years. As we move closer to the five-year mark, we must be certain we are making the prediction possible. Can we reach those predictions with our current education structure?

It is not a time to maintain status quo, we must prepare for the required changes. Using Spencer Johnson’s Who Moved My Cheese as an allegory, our profession is Cheese Station N, the maze is the trials it will take to reach our cheese. Laboratory professionals must assume the role of Sniff and Scurry and not be Hem waiting for things to be back like they were.

REFERENCE


Carol McCoy PhD CLS(NCA) is the Clinical Laboratory Sciences Continuing Education Editor.
The following letter was written to Susan Leclair, Editor-in-Chief.

Part of my lunch time reading today included the Winter 2003 issue of Clinical Laboratory Science. I just wanted to send a note of appreciation for your editorial. Just before lunch, I presided at one of my college’s Open Lab days, a recruitment tool where high school students tour the building, visiting the various health programs we offer. Our college has three big programs: nursing, dental hygiene, and radiography. Students tour FOUR programs, so even if they choose the big three, they have to visit CLS, or nuclear medicine, or respiratory care, or something else that may be better suited to their interests.

I had a small but very enthusiastic group this morning. I had set up microscopes with various blood smears and bugs to look at. We had some agar plates with bacteria, and an expired unit of RBCs to examine. I described how chemistry was highly automated and that we taught students how to use automation on campus. As they moved from station to station, the CLMA recruitment video played in the background.

It’s amazing how some of these enthusiastic students can turn into such depressing professionals. You are absolutely right about how many of us isolate ourselves in the lab, refusing to look at the big picture of healthcare except as something that threatens us. The students thought that doing the testing MIGHT BE fun. I often feel guilty that we spend so much time stuffing factoids into their heads that we don’t demonstrate how rewarding our jobs can be.

I am firmly in favor of licensure and professional recognition. You are absolutely right that we prevent our own success by trying to pass the perfect bill, or objecting to paying $50 a year for a license that would increase our incomes by several thousand dollars. I think we may need to work first on raising our self esteem. We ARE professionals, we DO contribute, and our work IS rewarding. Our recent grads do see themselves as part of the healthcare team. It concerns me that many of the practicing techs try to keep them from succeeding.

Barbara Ross MT(ASCP), Ferris State University, 200 Ferris Drive, Big Rapids MI 49307-2740
WASHINGTON BEAT

CLIA Regulations Updated

KATHY HANSEN, DON LAVANTY

On January 24, 2003, the Centers for Medicare and Medicaid Services (CMS) published revisions to the Final Rule that regulates clinical laboratories under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). The revisions to the regulations address substantive changes in quality control practices and in the qualifications for non-physician laboratory directors. They also include reorganization of the previous regulations into a format that should be more logical to follow, and removal of some duplicative provisions.

The revisions to the CLIA regulations have been expected for years, and CMS officials, in presentations at conferences and in written communication, had promised them “soon” for a long time.

To recap history, the Clinical Laboratory Improvement Amendments of 1988 (CLIA) were passed overwhelmingly by Congress in 1988, in response to public and media concerns about the quality of laboratory testing. There were media stories about misread PAP smears, inaccurate cholesterol testing, and concerns about unregulated laboratories’ performance. The original legislation was generally worded and declared the intent that testing would be reliable and accurate regardless of where it was performed. CMS (then the Health Care Financing Agency – HCFA) was authorized to write regulations to administer the law. Because of many concerns and comments about provisions of the first draft of proposed regulations, the first final rule, containing most of the provisions that we still practice under today, was published on February 28, 1992. Additional changes and extensions to deadlines or phase-in periods were published in final rules on December 6, 1994; May 12, 1997; October 14, 1998; and December 29, 2000.

The text of the January 24 final rule can be found at the Federal Register web site, www.access.gpo.gov/su_docs/aces/aces140.html. It is about 75 pages in length. When a federal agency publishes a final rule with comment period, at the end of that comment period it publishes a summary of how many comments were received, what their substance was, and why the agency either did or did not make changes in response to the comment(s). Much of the January 24 publication is devoted to the summary of comments and responses, and also to CMS’s estimate of the financial burden of the new regulations. Because of this, you may find the document somewhat repetitive to read.

The sections of CLIA have been significantly reorganized to consolidate duplicate statements, to distribute quality assessment requirements throughout the various sections, and to organize the regulations to mimic the flow of specimens through the laboratory, i.e., preanalytical, analytical, and postanalytical requirements. The new organization was recommended by the Clinical Laboratory Improvement Advisory Committee (CLIAC). There is a large (nine page) table in the Federal Register that ‘crosswalks’ sections of the ‘old’ CLIA to the ‘new’ CLIA. You will find this useful if you are familiar with or have marked where to find things in the old regulations and want to quickly find them in the new ones.

The new document is not the ‘final final’ rule, however. Given the rate of change in clinical laboratory science, it is perhaps doubtful that there can ever be ‘final’ regulations. In the current comments, CMS says, “We intend to publish a notice of proposed rulemaking addressing proficiency testing issues in more detail in the future”, and, “We intend to publish, at a later date, a rule specific to laboratory information systems.”

A terminology change is that, in many sections, moderate and high complexity testing are referred to as “non-waived” testing. The quality control requirements are now substantially the same for both levels of testing. In another terminology change, “quality assurance” has been changed to “quality systems” and “quality assessment” throughout. In addition, there are a number of examples where specifics of certain requirements have been removed from the regulations and included in Appendix C of the State Operations Manual.
(CMS Pub. 7), subpart M, instead. (This is the manual used by the state surveyors who do CLIA inspections on behalf of CMS.) This will allow for more timely updating of certain requirements. An example is the list of certifying boards that are acceptable to CMS for certifying PhD laboratory directors. Four specific boards were previously listed in the regulations. Now the list has been moved to the State Operations Manual and includes eight boards. The list of approved boards is also found at http://cms.hhs.gov/clia/dirclcon.asp.

The rule specifies qualifications for non-physician laboratory directors for high-complexity testing laboratories. The requirement as of Feb 24, 2003 will be a PhD with board certification by one of the specialty boards approved by CMS. There is a ‘grandfather’ provision for current PhD directors who are not board certified. The response that CMS made to commenters is interesting. “The few comments opposed to board certification indicated certification does not ensure the performance of individuals and that employee skill evaluation is the responsibility of the employer. These commenters also noted the absence of evidence documenting that certified individuals perform better than noncertified individuals.” CMS’s response is “Although certification does not provide absolute assurance that individuals will effectively fulfill the responsibilities required of directors, it is a recognized benchmark of competency and an appropriate mechanism for qualifying individuals to serve as laboratory directors. In addition, the ongoing continuing education required by each of the HHS-approved boards to retain certification helps ensure these individuals maintain a current knowledge base.” Note: CMS’s position on certification is consistent with what ASCLS has always endorsed for other laboratory personnel.

In the area of quality control, the American Society for Microbiology (ASM) was very successful in documenting extremely low failure rates for purchased media and stains, so that for many tests the new regulations call only for running quality control (QC) on new lots and shipments, rather than previous requirements for weekly checks. For some serological assays, QC at two levels is now required only once per 24 hours instead of with each run. In general, requirements for QC were eased in all areas except mycobacteriology, where the requirement for a negative control was added to the previous requirement for only a positive control for acid-fast stains.

Method validation will be required for any test system placed in service after April 24, 2003. This requirement has been in place for high complexity testing but will now be required for moderate complexity testing also. All tests must be evaluated for accuracy, precision, reportable range, and appropriateness of reference range for the laboratory’s patient population.

The section on the regulatory impact of the new requirements provides interesting updated (2001) figures on the CLIA laboratories. There were 171,010 total laboratories in 2001, of which 91,540 perform only waived testing and 38,304 do provider performed microscopics (PPM). These two categories comprise 76% of the total laboratories and are not affected at all by the changes in the regulations. The other 24% of the laboratories fall into two categories, 22,720 compliance laboratories (those that are inspected by CMS) and 16,124 accreditation laboratories (those that are inspected by an agency with deemed status such as CAP, AABB, JCAHO, and others). CMS estimates that the quality control and method validation changes will affect the compliance laboratories and the accreditation laboratories that have been accredited by COLA, a total of 29,601 laboratories or 17% of the nation’s total. The other accreditation laboratories are assumed to have already been held to more stringent requirements by their accrediting body.

Nevertheless, it would be prudent to take a look at how the new regulations will affect QC in your laboratory, especially for those who work in microbiology and related areas.

**ASCLS ANNUAL MEETINGS INFORMATION**

Forms for submitting program proposals and abstracts for future ASCLS Annual Meetings will be available on the ASCLS Web site under meeting information. The Web site address is: http://www.ascls.org/
Clinical Laboratory Educators Conference
2003 Abstracts

POSTER PRESENTATIONS
Authors listed in bold face type were the presenters.

Addressing Curriculum Problems through Creative Planning and Scheduling
Cheryl Burns MS CLS(NCA), Linda A Smith PhD CLS (NCA), Shirlyn B McKenzie PhD CLS(NCA), Ronald Holton PhD, Betty Dunn MS CLSp(CG), University of Texas Health Science Center at San Antonio, San Antonio TX.

As the knowledge base of the CLS profession grows and the role of the laboratory professional expands, educators are challenged with curricular problems of too much content and too little time. Most CLS curricula already exceed the credit hours required for a BS degree, with many students taking five years or more to complete the program. In our department, CLS faculty were faced with two problems: the need to expand the molecular diagnostics course content for three student groups (CLS, cytogenetics, and molecular diagnostics students) and the integration of an immunology course (formerly taught at another institution) with serology. This had to be done without increasing credit hours, eliminating basics, or spreading faculty ‘too thin’. The faculty held several brainstorming sessions to discuss integrating course content, determine student needs, and identify how the course could fit into the curriculum. The result was creation of a combined molecular and immunologic diagnostics course (lecture and laboratory) roughly divided into thirds. All three groups of students take five weeks of molecular diagnostics. The CLS students also take five weeks of immunology and five weeks of immunologic diagnostics. Multiple faculty teach the course depending on their area of expertise. Faculty agreed to give up turf and work together to fully integrate content. This course reorganization and development resulted in adding new content without adding credit hours. In addition, students gained communication and teamwork skills by working together on projects.

Biodefense Awareness Courses – Addressing Personnel Shortages and Preparedness for Biological and Chemical Attacks
Pat Greenup PhD MS MPH, University of Alabama at Birmingham, Birmingham AL.

The focus of bioterrorism preparedness has been the front line workforce in public health and medical emergency practitioners. Awareness and performance objectives have been identified for first responders with the ultimate goal to achieve a competent workforce able to respond to bioterrorism and other current and emerging health threats. A major limitation to national, state, and local biodefense preparedness is the current and emerging manpower shortages across the health professions. One proactive strategy is the offering of biodefense related courses to undergraduate and graduate students. The primary objective is to make awareness level information available and to recruit students into the health sciences for future response to incidents involving weapons of mass destruction. Two course offerings (a two credit course and a three credit Honors Program course) present awareness objectives including terrorism, event types and recognition, detection systems, and key competencies needed for biological and/or chemical response. Core content areas are linked to the WHO definition of health and the identification of interdependencies between social, psychological, political, economical, technological, legal, and ethical issues related to biodefense, counterterrorism, and emergency preparedness. The instructional design for these elective courses offered by the UAB Clinical Laboratory Sciences Programs will be described. An evaluation of the first course offerings and the potential for recruiting students to health professions, life sciences research and public health programs will be described.
The Clinical Laboratory Practitioner: A New Level of Practice
David G Fowler, PhD CLS(NCA), Tina Martin MSN FNP, University of Mississippi Medical Center, Jackson MS.

Over the past thirty years many health professions have evolved into an expanded scope of practice while the CLS profession has seemed to languish. Recently there has been a task force initiated to study the feasibility of the post-baccalaureate entry level into the profession. Some health professions have limited success with requiring a post-baccalaureate entry-level while others have used an alternative education model to expand their scope of practice. This study is designed to look at potential areas of practice for the CLS and propose a graduate curriculum to prepare the practitioner for an advanced level of practice. The study identifies six issues that must be addressed before implementation of the advanced level of practice. These include patient safety, cost/benefit analysis, patient clinical outcomes, professional liability, healthcare reimbursement, and licensure/certification. Before entering into a new level of practice the CLS would be required to obtain an educational level commensurate for the anticipated level of practice. The proposed curriculum model is adapted from established healthcare educational models and culminates in a professional doctorate. This study is designed to address the issue of post-graduate education for the CLS profession and offers an alternative to the entry-level master’s degree.

Clinical Testing Via University Website for Off-Campus Students
Libby M Spence PhD CLS(NCA), Thomas B Wiggers MS, University of Mississippi Medical Center, Jackson MS.

While current shortages in CLS have increased student class sizes, clinical facilities for training have remained scarce. To provide clinical internships for all students it becomes necessary to contract with outlying facilities for training. To offer off-campus students the same accessible, comparable clinical testing as those on campus, an on line testing program, TestGenerator®, was utilized to provide Web testing.

The only requirement for any clinical facility to make testing available is an Internet connection as the program uses any browser and any operating system. Many security features, such as a password-protected page, random generated passwords, print and browser functions are not accessible from within the test, and the URL of the examination file is not visible, are employed. The student while being monitored, logs on, takes the test, and is immediately able to view his or her score. Simultaneously, the university clinical coordinator receives an e-mail of the student’s score and a test analysis to review individually with the student at a later date. This method has proven to be valuable to students, faculty, and clinical facilities in a number of ways. A high level of satisfaction exists for all parties involved because the program is easy to use and administer and provides immediacy of results for both student and faculty member. This practice relieves the clinical personnel from the responsibility of generating exams.

Comparing International Laboratory Education
Linda J McCown MS CLS(NCA), Jewish Hospital College of Nursing and Allied Health, St Louis MO.

Educators and employers of laboratory personnel face a difficult situation when trying to assess applicants from other countries. Internationally, medical laboratory science not only lacks a standardized educational track, it also lacks uniformity in the levels of practice within the profession. Professionals who may be licensed and credentialed in their own country may not be allowed to practice in another country. Through phone interviews, electronic mail, and literature searches, information was gathered about the education and credentialing of medical laboratory personnel in Australia, China, Ethiopia, and the Philippines. Like the United States, Australia has multiple routes to employment in a medical laboratory. Australia has programs ranging from two to three year technical education to a doctorate in science. Employment is dependent upon membership/registry with the Australian Institute of Medical Scientists. The Peoples Republic of China also has a hierarchy of positions in the medical laboratory and a variety of ways to become a medical laboratory technologist. Employment depends upon completion of a formal program. Ethiopia has one bachelor’s degree program and three two-year technician programs. Laboratory professionals are registered and licensed by the Ministry of Health and an individual’s place of employment is determined by the Ministry. In the Philippines, the only level of education is the four-year bachelor’s degree which is followed by an examination of the Board of Medical Technology (governmental). Hopefully this study will increase awareness of the variety of laboratory education around the world and the difficulty in comparing education and credentials.
Expanding Curriculum: A Forensic Science Concentration within a CLS Degree

*Julie A Hammerling MS CLS(NCA), Jo Ann Wilson PhD CLDir(NCA)*, Florida Gulf Coast University, Fort Myers FL.

A surge of interest in forensic science has swept the nation as live courtroom dramas, fictional television episodes, and written media highlight laboratory science in criminal detection applications. Best poised for integration of this applied laboratory science into existing curriculum are the clinical laboratory science (CLS) programs across our nation. Currently, very few academic programs exist that provide a course of study in forensic laboratory science. CLS faculty possess the laboratory skills and theoretical basis behind forensic laboratory courses providing the perfect marriage of the two curricula. CLS students develop the skills and knowledge for clinical laboratory application that are easily transferred to forensic laboratory analysis. Florida Gulf Coast University developed a forensic science concentration within the CLS baccalaureate degree, with support of the existing university criminal justice program. Students choose forensic laboratory science or CLS or elect to combine the concentrations with additional coursework within their degree. The curriculum integrates a theoretical basis and applied technology of crime laboratory analysis balanced with laboratory science and criminal justice understanding. Courses include Forensic Microscopy, Human Genetics, Biochemistry, Molecular Genetics and Diagnostics, Forensic Analysis, Forensic Toxicology, Courtroom Forensics, and an Internship. The curriculum is augmented with courses from criminal justice including Criminal Justice Systems and Procedures, Forensic Psychology, Advanced Forensics, Constitutional Criminal Law, and Drugs, Alcohol, and Crime. As universities continue to place student numbers as the marker for successful programs, adding concentrations that attract students and meet the needs of today’s marketplace is essential. Adding a forensic science concentration to your existing baccalaureate degree is a positive and cost-effective alternative.

Evaluation of Small Group Testing Strategies in a Clinical Microbiology Course

*Mary F Lux PhD CLS(NCA)*, University of Southern Mississippi, Hattiesburg MS.

Lecture has been the traditional delivery method for content-driven courses such as clinical microbiology. The introduction of group activities into a structured, traditional lecture course represented a shift in style for both the instructor and students. Group activities help students to develop active learning habits and to cooperate to achieve a common goal. Classroom learning activities for small groups included preparation of illustrations, development of in-class questions and responses, solution of case study problems, and presentations of books or journal articles. If students invest in group learning activities, it follows that groups should participate in evaluation or assessment. The group evaluation techniques included occasional group quizzes and the case study portions of three major exams. The performance on group quizzes produced higher average grades when compared with the average grades from years in which each student completed the questions as individuals. Likewise, performance on the evaluation of the case history portion of the exams was higher than with the individual efforts of previous classes. However, overall course grades were not considerably higher for classes with group activities as compared to classes in which each student worked alone. Nevertheless, all students in the classes with group activities reported high satisfaction with the group’s activities and testing strategies.

Human Granulocytic Ehrlichiosis: A Case Study

*Brenda L Bouchard MS CLS(NCA), Lynne A Brodeur CLS (NCA)*, Department of Medical Laboratory Science, University of Massachusetts Dartmouth, Dartmouth MA.

Ehrlichioses are intraleukocytic bacteria of the family *Rickettsiaceae*. The bacteria are reported to be spread through contact with ticks. Ehrlichiosis symptoms can range from asymptomatic to severe life-threatening conditions. Although rarely seen in clinical practice, laboratorians working in high tick exposure areas should be aware of the laboratory results associated with ehrlichiosis. During a summer evening, at a Cape Cod hospital in Massachusetts, a 68 year-old male sought treatment for fatigue and shortness of breath. He had received chemotherapy recently and believed that his symptoms were probably due to the treatment and weather. Also, he stated that he noticed an insect bite on his left hand. A CBC count demonstrated a WBC count of 12.8 x 10⁹/L, reference range 4.8-11.2 x 10⁹/L. A peripheral blood smear demonstrated 12 bands, 1 metamyelocyte, 4 lymphocytes, 1 monocyte, 2 eosinophils, 0 basophils, and 80 neutrophils with occasional hypersegmentation. The following morning the WBC count was 1.4 and the smear presented 2 bands, 44 lymphocytes, 1 monocyte, 2 eosinophils, and 80 neutrophils having prominent hypersegmentation and inclusions. These findings prompted a review of the smear by a hematopathologist. On the third day, the patient’s WBC...
flagged a delta message of 0.5 (from 1.4; 12.8). The smear revealed 1 neutrophil, 1 band, 5 metamyelocytes, 2 myelocytes, 80 lymphocytes, 6 monocytes, and 5 eosinophils. Differential diagnosis from the pathologist confirmed intraleukocytic ehrlichiosis within the neutrophils. Using immunofluorescence the human granulocytic ehrlichiosis (HGE) antibody was detected, supporting the cellular morphological findings and confirming the diagnosis of HGE transmitted via the patient’s insect bite.

In Search of a Meaningful Senior Honors Research Project
Margot Hall PhD FAIC CChem (MRSC), Sabrina Bryant MS CLS(NCA) MT(ASCP), Jane Hudson PhD CLS(NCA) MT(ASCP) SM, Mary Lux PhD CLS(NCA) MT(ASCP), Carol Beck EdD, MT(ASCP) SBB, Shelley Myers MT(ASCP), The University of Southern Mississippi, Hattiesburg MS.

The problem addressed in this study was how to design and execute a senior honors research project which would be meaningful to medical technology students while also meeting university requirements. The overall goal was to introduce the students to applied chemistry research. Criteria for the research problem included: 1) it must be important scientifically; 2) it must introduce the student to a body of medical/scientific literature, research design, state of the art laboratory techniques, and statistical treatment of data; 3) it must culminate in publishable data; 4) it must require less than one year for completion (not open-ended); 5) it must involve the use of available and affordable resources; and 6) it must culminate in a written document which meets university standards for honors theses. In addition to the writing and defense of his/her senior honors thesis, each student was required to submit an abstract and give a presentation at a regional meeting. Sources of funding, instrumentation, reagents, and specimens are discussed. The work resulted in the evaluation of ten different serological tumor antigens (CA19-9, CA195, CA50, CA72-4, CA125, CA15-3, CA27.29, CEA, AFP, and Cyfra21-1) for their diagnostic efficacy in a population of 554 patients plus 200 healthy adults. Diagnostic predictive values were generated for breast, pancreatic, and gastric cancers and those results are discussed. The study resulted in three senior honors theses, four papers (two national, two regional), five presentations with abstracts (two national, three regional), and four student awards (two national, two regional), thus meeting all goals.

Impact of a Change in CLS Program Structure on Student Retention
Karen R Murray PhD CLS(NCA), Tarleton State University, Fort Worth TX.

Retention is a critical factor in capacity enrollment programs. Students not retained represent both a financial loss to the program in tuition revenue and the loss of a new professional in a time of nationwide shortage of clinical laboratory professionals. Program assessment reports had revealed retention rates that were determined to be unacceptable when compared to program goals. A four-part plan entitled Strategies and Objectives for Success (SOS) was developed to improve student success. The first part involved a complete redesign of the CLS program structure and was implemented as a tool for increasing student retention rates, and thus success in the academic portion of the program. A new structure was adopted in which the CLS program courses were redesigned to divide the program into distinct phases. The phases were graduated in content difficulty and outcome objectives, resulting in a learning environment more conducive to student success. This study uses student retention rates to evaluate the first part of the SOS plan. Retention rates were compared using six different classes; three classes prior to the change in program structure and three after the change was implemented. Comparison of retention rates between the two groups revealed that retention increased by eight percent. These results were encouraging and as a result our program plans to implement the next three parts of the SOS plan. Other CLS programs with retention problems may benefit from adopting a similar structure.

Implementation of Manipulatives in the Clinical Microbiology and Immunology Classroom
Beverly Barham PhD, Illinois State University, Normal IL.

1) The ‘hands on’ experience for CLS students in the microbiology and immunology classroom is an essential part of the learning process. The cost of that experience can be a barrier for many programs. 2) Implementation of manipulatives can help students understand the basics of both traditional and molecular diagnostic testing methodologies without spending resource dollars. Manipulatives can be designed by students using common items such as construction paper or posterboard. Through creative but simple design, the basic components needed for a testing method-
CLINICAL PRACTICE: EDUCATION

ology can be made. The students can then take these designs, apply the theoretical concepts of different methodologies such as enzyme linked immunosorbent assays and create a very simple but effective visual ‘hands on’ educational tool. 3) By using manipulatives, students were able to solve case studies and identify possible sources of errors in testing methodologies correctly in 80% of the challenges (n = 20) without any additional help. Students were also challenged to design testing strategies for solving a particular problem in groups or individually which allowed for enhanced student engagement. In a traditional clinical immunology course, the implementation of manipulatives throughout the semester was mentioned as a positive learning experience in 75% (n = 14) of the course evaluations. 4) Implementing manipulatives can contribute to a positive student learning experience and save resource dollars. It can also help assure that the next generation of CLS students will have a strong foundation in both theory and application of laboratory testing methodologies as they enter the profession.

Increasing Enrollment with Online and Off-Campus Delivery
Valerie Polansky MEd MT(ASCP), St Petersburg College, St Petersburg FL.

When single-digit enrollment threatened the future of the Medical Laboratory Technology Program at St Petersburg College, the curriculum was redesigned so that all of the courses in the major could be completed off campus. It was hypothesized that through distance education, program accessibility would be improved and enrollment would increase. The infrastructure to support a distance program was already in place at the college as a result of a five million dollar multi-year federal grant designed to increase access to workforce training. The program director and four adjunct instructors converted the theory courses for delivery in WebCT and redesigned the laboratory courses so that they could be carried out in affiliated clinical laboratories. The conversion was completed in two years. A national marketing campaign was undertaken and the new program was launched in August 2001. In the program’s first year, 20 students matriculated and new affiliation agreements were developed with six clinical laboratories in distant locations. By September 2002, requests for information about the program had been received from 680 prospective students and 57 laboratory directors in 48 states. These preliminary data support the hypothesis that program enrollment can be increased through distance education. The significant level of interest in the program throughout the country suggests that this model of education could revitalize other programs and help to ameliorate the personnel shortage. The program’s first distance education class will graduate in May 2003. At that time a comparison of outcomes for the distance and traditional programs will begin.

Investigating Medical Laboratory Practitioners Scope of Practice
Donna Surges Tatum PhD CAE, Kory Ward-Cook PhD MT(ASCP) CAE, ASCP Board of Registry, Chicago IL.

Job task, or practice, analyses are a vital component of the certification process. They are used to validate examinations by providing a link between job performance and examination content. Before an examination is developed, and every three to five years thereafter, a job task analysis must be performed to define or confirm the scope of practice in order to assure appropriateness and relevance of the certification. In 2001, ASCP Board of Registry staff visited 22 diverse practice settings and recorded more than 150 observations to create an ethnographic report. These data were used to develop an in-depth Job Task Survey for Medical Technologists (MT), Medical Laboratory Technicians (MLT), and Phlebotomists (PBT), mailed in early 2002. The table below shows the response rate is more than sufficient to determine the various scopes of practice and the validity of the certification examinations.

<table>
<thead>
<tr>
<th>Category</th>
<th># Mailed</th>
<th># Returned</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>17,310</td>
<td>2,665</td>
<td>15.4%</td>
</tr>
<tr>
<td>MT</td>
<td>7,010</td>
<td>1,058</td>
<td>15.1%</td>
</tr>
<tr>
<td>MLT</td>
<td>5,626</td>
<td>876</td>
<td>15.3%</td>
</tr>
<tr>
<td>PBT</td>
<td>4,674</td>
<td>731</td>
<td>15.6%</td>
</tr>
</tbody>
</table>

Results indicate overlapping scope of practice with MTs and MLTs for many tasks. However, MTs perform complex tasks more often than do MLTs. PBTs have defined jobs and do not practice outside clearly defined parameters. Other variables examined, such as geographic differences, length of time in the profession, practice setting, type of facility, and schedule, are useful for blue printing the examination and validating the examination content. Further, educators can review their curricula in light of practice, and government agencies will have the facts when determining laboratory regulations.
Investigation of the Utilization of Learned Generic Skills by CLS/MT Practitioners

H Jesse Guiles EdD, Michelle Brown, University of Medicine and Dentistry of New Jersey, Newark NJ. Donna Surges Tatum PhD, Psychometrician ASCP-BOR, Chicago IL.

Application of generic skills such as: Analytical Reasoning, Correlation of Data, Communication, Computer Use, Decision-Making, Precision Studies, Problem Solving, Quality Assessment, Research, Supervision, Teaching, Technical Writing, Troubleshooting, and Utilization Studies is considered a hallmark of competent baccalaureate level CLS/MT practitioners. In 2002, Guiles (CLS15:23-9), looked at the acquisition and utilization of these skills by a cohort of CLSs/MTs who stated they left the field (LTF). It was proposed that the same skills should be examined for CLS/MTs who remained in the field (ITF). Data were collected from 517 (44% response rate) practitioners who have been ITF since 1993. Utilization was grouped into two categories: used, didn’t use. Learning was grouped into three categories: learned as a student and developed as a practitioner, learned as a practitioner only, never learned. Chi square analysis showed a significant difference in learning vs. using the skills in ITF jobs for computer use, research, supervision, problem solving, and utilization studies. Except for learning computer skills, no significant difference in learning or using the skills in ITF jobs was observed between those practitioners who qualified for the ASCP-BOR certification exam as NAACLS program graduates vs. other routes. These findings are in contrast to the LTF study that showed no significant differences in using vs. learning these skills and several significant differences in learning the skills via different eligibility routes. These findings may indicate that LTF jobs provide graduates with better opportunities for applying certain learned generic skills than do ITF jobs.

Demonstration of Proper Micropipetting Technique, Calculation of Precision and Accuracy, and Beer’s Law

Wayne Gade PhD CLS, University of Illinois at Springfield, Springfield IL.

The Science Division at UIS has upper division programs in biology, chemistry, and CLS and attracts traditional and non-traditional transfer students from central Illinois who vary greatly in laboratory training and expertise. A laboratory techniques course is required of all students to insure that students gain essential proficiencies. This abstract describes a laboratory exercise that provides substantial pipetting practice, and generates data that illustrates principles of precision, accuracy, and Beer’s Law using a 96-well microtiter plate and EIA plate reader. Students develop pipetting technique while filling three columns of the plate with specified volumes of dye, and they also generate sets of eight repetitions used to calculate precision (typically 2.5% CV). Students perform a series of two-fold dilutions, construct a standard curve to illustrate Beer’s Law (linear relationship of absorbance to concentration), and determine concentrations of unknowns. Students also construct an atypical standard curve, by adding increasing volumes of dye (from 50 mL to 300 mL), which demonstrates linearity between absorbance and path length or volume. Finally, the microtiter plate is re-read at another wavelength (and different pH of dye), illustrating that molar absorptivity is constant only for specified conditions, and that changes in conditions affect absorbance (even with constant concentration and path length). Results on final practical exams demonstrated improvement in pipetting competency and a better understanding of Beer’s Law.

Survey of Clinical Instruction of Pathology Residents in Immunohematology

Linda M Hawthorne MHS MT(ASCP)SBB, Lynda Britton PhD, Debra Judd PhD, Aixa Garcia MD, Diana Veillon MD, Deborah McCaskill MT(ASCP)SBB, Louisiana State University Health Sciences Center, Shreveport LA.

This study investigated methods of instruction used in clinical pathology (CP) programs to train residents in immunohematology. The exam scores of residents suggested our current protocol might inadequately train residents in basic immunohematology concepts and techniques. Time, budget, and staffing constraints make using a supplemental computer tutorial an alternative to expanded traditional training. Applications of computer assisted instruction (CAI) show promise of fostering student enthusiasm and learning, while reducing instructor time and participation. United States CP programs were surveyed to determine training methods and gauge interest in incorporating a hypermedia tutorial. Teaching personnel at 110 randomly selected CP programs were surveyed and statistics were used to analyze data. A total of 60/110 surveys were returned (54.5% response rate). Respondents from 47/58 programs (81%) considered their trainers experienced teachers. Medical technologists performed the majority of bench instruction at 50/59 sites (84.7%). Independent reading and lecture were used primarily in 53/60 programs (88.3%). Although only 2/60 (3.3%)
currently used CAI, 53/59 (89.8%) expressed interest in incorporating CAI. Respondents assessed training effectiveness as excellent in 19/59 programs (32.2%), good in 30/59 (50.8%), and satisfactory in 9/59 (15.3%). A primary research goal was comparison of our CP resident immunohematology training protocol to that of other programs. Results indicate our training program is comparable to other respondents. Independent reading, lecture, and bench training are the primary methods of instruction. Appreciable interest exists in incorporating CAI into training. Data provides useful information to staff for assessing their training protocols and should facilitate streamlining or redesign.

Updating Pre-CLS Requirements in Molecular Biology and Biochemistry to More Effectively Prepare Students for New Molecular Technologies

Mary Louise Greeley PhD, Salve Regina University, Newport RI.

The techniques of molecular biology are increasingly being used in the clinical laboratory both to diagnose patients and to monitor their response to treatment and/or the progression of their disease. Because of the increased use of molecular diagnostic techniques, many clinical laboratory internship programs are fast revising their curricula. However, many students come to the clinical year with a poor background in biochemistry/molecular biology making it harder for them to understand the applications of these techniques as presented during the clinical practice. Since most CLS programs require either biochemistry or organic chemistry, the content presented in organic courses was compared to that in biochemistry courses to see which would better prepare students for the 21st century clinical laboratory with its increased emphasis on molecular techniques. The biochemistry courses contained the theories and techniques of nucleic analysis and structure while the organic chemistry courses did not, making biochemistry a more appropriate prerequisite. A survey of the undergraduate requirements for biology majors revealed that many colleges/universities have either dropped their requirements for organic chemistry or reduced this requirement to one semester. This change in biology requirements coupled with the fact that biochemistry courses provide a better foundation for meeting the new demands of clinical molecular biology suggests that a two semester preclinical biochemistry sequence should be required for all CLS students.

The WEBCLS Project: Its Effects on Participating CLS Faculty

Vicki S Freeman PhD, David Holcomb EdD, University of Texas Medical Branch, Galveston TX.

The Web-based Education in Clinical Laboratory Sciences (WEBCLS) project, in addition to the overall goal of developing courses for CLS distance education, had additional objectives including improving participating CLS educators’ skills in designing, developing, delivering, and evaluating interactive, Web-based instructional programs. To assess the project’s outcomes in accomplishing this objective, a survey was sent to the 27 primary faculty participants in WEBCLS activities. Twenty-four participants completed the survey for an 89% response rate. Overall, the WEBCLS project accomplished its objective of improving CLS educators’ Web-based, distance education course development skills through systematic instructional design training. One of the most positive outcomes was the belief that their participation in the project expanded their contacts with colleagues in CLS education as well as with instructional design experts, computer programmers, and other technical support personnel. This outcome prompted the faculty to report that this enhanced collegial relationship will sustain their interest in curriculum development over time. In addition, the CLS faculty members reported that they now integrate more technology in their teaching, better organize their courses, develop and use alternative methods of evaluating students, and were moving away from lectures towards more student-centered activities. Problems associated with faculty participation in the project primarily focused on lack of quality time devoted to developing the CLS instructional units. Heavy teaching loads and lack of release time caused delays and gaps in the time available for project activities of most faculty participants. The WEBCLS project clearly had positive effects on the participating CLS faculty, and, subsequently, should enhance CLS education.

When the Road Becomes Bumpy, Throw It Some Curves: Making the Transition from Live Lectures to Interactive Web-based Courses

Wendy L Arneson MS CLS(NCA), Michelle S Kanuth PhD CLS(NCA), University of Texas Medical Branch, Galveston TX.

With the shortage of laboratory personnel, Web-based instruction is becoming necessary to expand options in educating clinical laboratory scientists (CLSs). Web-based courses can meet the needs of place-bound students who are
not able to attend regularly scheduled lectures in a course. Such instruction is ideally interactive; just such instruction was designed for courses in chemistry and pathogenic microbiology to be delivered to clinical laboratory technicians (CLTs) in a CLT to CLS articulation program. Unfortunately, these courses were not available in the on-line format in time for use during the first designated semester. To meet a variety of learning styles and still meet the needs of distance students, an array of formats for delivering the learning materials were used in these courses. Didactic material was delivered both video-streamed and in PowerPoint with expanded verbiage or narration. Extensive study questions and case studies were also provided to these students. Students answered and discussed these materials on the discussion board. Prompt instructor feedback via e-mail and the discussion board was provided. Students in the Web sections of these two courses performed as well as the students in the on-campus sections on examinations and assignments. For educators lacking the availability of interactive Web materials, this approach provides the opportunity to educate students via the Web with minimal investment in a Web-authoring program. Blackboard, along with other similar programs, is very intuitive and easy to use. Place-bound students that may have been previously unreachable can now be served in this Web-based structure.

TECHNOLOGY DEMONSTRATIONS

Antimicrobial Susceptibility Testing: An Interactive Educational CD-ROM

Judy R Delany MPH MS MT(ASCP), Janet F Hindler PhD, Fred C Tenover PhD, Eunice R Rosner EdD MT(ASCP), Centers for Disease Control and Prevention, Atlanta GA; Diana Mass MA CLS(NCA), Arizona State University, Tempe AZ.

Antimicrobial susceptibility testing is becoming increasingly complex as new antimicrobial agents are introduced and bacteria develop new resistance mechanisms. These changes present challenges for the clinical laboratory in testing and reporting. This continually changing field also presents a challenge to educators preparing tomorrow’s CLTs. An interactive CD-ROM, available free of charge, has been developed which is designed to meet these challenges. This program, developed by leading experts in the field of antimicrobial susceptibility, is composed of four different modules: modes and mechanisms of action, testing methods, Gram-positive organisms, and Gram-negative organisms. Continuing education credits and continuing medical education credits (CEUs and CMEs) may be earned for each module independently. The course answers such questions as: 1) How do antimicrobial agents work? 2) What organisms should I test? 3) What antimicrobial agents should I test? 4) What methods should I use? and 5) How should I report results? The course is structured with basic components, which could be used in the education of the beginning CLS student and also includes very detailed explanations of complex modes and mechanisms of action of antimicrobial resistance by different organisms, which would be of interest to experienced microbiologists.

Computer Simulated Laboratory: Student Perception and Outcomes

Janelle M Chiasera MS, Sally V Rudmann PhD, The Ohio State University, Columbus OH; Bob Harr, Bowling Green State University, Bowling Green OH.

Technology has significantly impacted our lives on several levels and has resulted in change almost on a daily basis. The students we are educating have grown up with technology and are used to an environment that is fast-paced, convenient, and available to them at their fingertips. As educators, we need to take our education environment to a level that will foster that technological environment and suit the needs of our students without sacrificing the quality of education. In an effort to accomplish this we developed a computer simulated blood gas module to study the effect of a computer simulated laboratory on student outcomes and perceptions. Clinical laboratory science (CLS) students from three university CLS programs were selected and randomly assigned to two treatment groups: computer simulation or traditional wet laboratory. The students completed a multiple-choice post-test and a questionnaire. A two-way analysis of co-variance was used to compare the scores on the cognitive post-test and descriptive statistics were run on the qualitative questionnaire.

With the population of students studied, we found no significant difference between institutions in post-test scores implying that the computer module worked as well as a traditional wet laboratory at all the institutions we studied. In addition, students seemed to have overall good perceptions of the computer laboratory as well as the traditional wet laboratory with the only difference being that the computer users felt they had more opportunities to assess their learning throughout the laboratory as compared to the wet laboratory users.

We believe that the use of technology in our laboratories will give us the opportunity to meet the needs of our stu-
Clinical Practice: Education

Students and lower the cost and time associated with our traditional laboratories without sacrificing the quality of education. In addition, it will allow us the ability to provide open access to our laboratories not only to our students, but to students at a distance as well as students in other health related disciplines that rely heavily on laboratory data, e.g., circulation technology and respiratory therapy students.

Computerized Atlas of Peripheral Blood Smears as a Job Aid
Michelle Montgomery CLS(NCA), Kathy Doig PhD CLS(NCA), Brian Winn MS, Michigan State University, East Lansing MI.

This demonstration will showcase a computerized atlas of peripheral blood smears to be used as a job aid by practicing clinical laboratory scientists (CLSs). Current computerized hematology atlases are designed for varying target audiences, most often novices, resulting in a number of shortcomings for optimal CLS use in the workplace. A needs assessment indicated general support for a job aid such as this one. The atlas, uniquely designed to support performance of white blood cell differentials, is intended to assist generalist staff in smaller laboratories and on minimally staffed shifts. A large number of digital images of peripheral blood smears are included. A database of text accompanies the images on an interactive CD-ROM. The atlas has been pilot tested for accuracy of content and usefulness in the workplace. Pilot test results have provided valuable information and suggestions that are currently being incorporated into the final product, which will be available for examination.

UND/Mayo Cohort; Putting it all Together
Susan Kante MS MT(ASCP) CLS(NCA), Nasser Hammami MS, University of North Dakota School of Medicine and Health Science, Grand Forks ND.

The University of North Dakota School of Medicine and Health Science, Grand Forks ND has entered into a cohort project with the Mayo Clinic, Rochester MN. The cohort program allows non-CLS Bachelor degree students to complete a CLS certificate (4+1) and MLT employees to complete a BS degree in CLS (2+2). UND also provides a CLS graduate program for employees. The cohort program allows students to complete didactic course material online, asynchronously. Intense laboratory sessions will be held at the Mayo Clinic in three, two-week blocks to minimize employee absence from work. The challenge becomes how to utilize technologies available to deliver courses, advise students, and maintain adequate communication. Blackboard.com, streaming audio/video, interactive online testing system, interactive delivery of the material on CD/DVD, and H.323 multiple site video conferencing are currently utilized to deliver the course material. A secure online database, handheld devices, and a document handling system are utilized to advise students and provide an effective interface for counseling at either location. This technology presentation will include examples of the above. The UND/Mayo cohort began the fall semester of 2002. Evaluation will consist of ongoing student interviews, student satisfaction surveys, faculty and student course assessment, and Mayo administrative review. The attrition rate and course completion rate will be monitored for students participating in the project. Results from the 2002 fall semester will also be presented.

Using Basic Technology to Prepare Supplemental Urinalysis Study Guide CDs
E Camellia St John MEd MT (ASCP) SBB, The University of Texas School of Allied Health Sciences at Galveston, Galveston TX.

Rapidly advancing computer technology provides opportunities to supplement course material requiring visualization of structures. Increasing numbers of adult learners with work and family responsibilities create a need for study sets that each student can copy and use as his/her schedule permits. Purchasing these materials commercially can strain the budget and preparing such materials can appear overwhelming.

In the CLS Urinalysis and Clinical Laboratory Methods courses for Physicians Assistants and Nurse Practitioners, study sets providing additional educational materials were needed. To resolve this problem, previously taken departmental slides plus photos captured using a digital camera mounted on a microscope mounted and linked to a computer were imported in Adobe Photoshop. Photoshop allowed sharpening of images using the contrast and intensity settings. The color contrast modified images to more closely reflect the sediment as it would be seen under the scope. These pictures were transferred onto PowerPoint slides, where structures of interest were identified and a brief narrative explaining the significance of the objects as well as hints for use in identification were included. Rather simplistic CDs were cost effectively produced to provide students additional learning opportunities to enhance their identification skills.
Trans Type Genotype Alpha Thalassemia Trait: A Case Study

ANGELA B FOLEY, LOUANN W LAWRENCE

ABBREVIATIONS: CBC = complete blood count; IDA = iron deficiency anemia; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; TIBC = total iron binding capacity.

INDEX TERMS: Thalassemia.

Clin Lab Sci 2003;16(2):79

Angela B Foley MS CLS(NCA) is Associate Professor, Louisiana State University Health Sciences Center, New Orleans LA.

Louann W Lawrence DrPH CLSpH(NCA) is Professor and Department Head, Louisiana State University Health Sciences Center, New Orleans LA.

Address for correspondence: Angela B Foley, Department of Clinical Laboratory Sciences, School of Allied Health Professions, Louisiana State University Health Sciences Center, 1900 Gravier Street, New Orleans LA 70112, (504) 568-4276, (504) 568-6761 (fax), afoley@lsuhsc.edu

A 22-year-old Caucasian female clinical laboratory science student agreed to donate several anticoagulated tubes of her blood to be used for analysis in the hematology student laboratory. She was in apparent good health and had no known history of a hematological disorder. Since manual white blood cell and platelet counts were being performed that day, her blood was run on an automated instrument so that the students’ values could be checked for accuracy against the instrument values. Laboratory results (Table 1) and peripheral blood smear (Figure 1) are shown.

This complete blood count (CBC) was done on an instrument which employs impedance and pulse editing technology in measuring the mean corpuscular volume (MCV). This technology has been reported to result in a ‘clamped mean corpuscular hemoglobin concentration (MCHC)’ which may not accurately reflect hypochromia. Hypochromic cells are more deformable and therefore present a smaller cross sectional profile as the cells pass through the aperture to be counted and sized. This results in an MCV that is reported as slightly lower than it actually is. Since the hematocrit is calculated by multiplying the MCV by the red blood cell (RBC) count, the hematocrit value will also be falsely decreased and therefore the MCHC will be somewhat overestimated. The net result is a slightly decreased sensitivity of the MCHC to the presence of hypochromia.1 However, a repeat CBC performed on a second instrument which employs pulse editing and hydrodynamic focusing

Table 1. Laboratory data

<table>
<thead>
<tr>
<th>Test</th>
<th>Case results</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count</td>
<td>5.30x10¹²</td>
<td>4.0 - 5.2 x 10¹²/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>124</td>
<td>120 - 160 g/L</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.388</td>
<td>35 - 46 L/L</td>
</tr>
<tr>
<td>MCV</td>
<td>73.1</td>
<td>80 - 100 fL</td>
</tr>
<tr>
<td>MCH</td>
<td>23.5</td>
<td>26 - 34 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>321*</td>
<td>310 - 370 g/L</td>
</tr>
<tr>
<td>Platelet count</td>
<td>204 x 10⁹</td>
<td>130 - 400 x 10⁹/L</td>
</tr>
<tr>
<td>RDW</td>
<td>13.2</td>
<td>11.1 - 14.5</td>
</tr>
</tbody>
</table>

Suspect flags: 3+ microcytosis, 2+ hypochromia*

Hemoglobin electrophoresis: normal

* Although the MCHC was within normal limits, the instrument suspect flag indicated 2+ hypochromia

Figure 1. Peripheral blood smear showing microcytic, hypochromic red cells (Wright’s stain, x 1000)
reported a comparable MCHC of 32.4 g/dL. Hydrodynamic focusing has been reported to improve the clinical usefulness of the MCHC. On manual review, the technologist reported 1+ microcytosis and 1+ hypochromia.

QUESTIONS TO CONSIDER
1. What are the possible causes for the decreased MCV and MCH?
2. What initial tests should be performed to attempt to determine the cause of the apparent microcytosis?
3. What additional special tests might be indicated?

The findings of microcytosis and hypochromia may be associated with iron deficiency, thalassemia, hemoglobin E, anemia of chronic disease, and sideroblastic anemia. Of these, the two most common causes are iron deficiency and thalassemia. Iron studies including serum iron, ferritin, and total iron binding capacity (TIBC) levels along with the red cell distribution width (RDW) can be used to help differentiate these disorders. A comparison of test results is found in Table 2. Also included in the table are results of iron studies performed on this case. It is apparent from these results that this was not a case of iron deficiency anemia (IDA). The normal results also exclude a diagnosis of sideroblastic anemia in which the percent saturation of transferrin would be elevated. The student was in good health and had no evidence of a chronic disease process. Hemoglobin electrophoresis was performed and the results were normal. From this information, a form of α-thalassemia was suspected.

THALASSEMIA
In the normal adult the majority of hemoglobin present, approximately 97%, is hemoglobin A. Hemoglobin A is composed of two alpha globin chains and two beta globin chains (α₂β₂). Other hemoglobins normally present include hemoglobin A2 (α₂δ₂) about 2%, and hemoglobin F (α₂γ₂) about 1%. Thalassemia is an inherited disorder in which there is decreased or absent production of one or more of the globin chains. This reduced production of globin leads to the formation of microcytic, hypochromic red cells. Alpha and beta globin genes are located on chromosomes 16 and 11 respectively. Thalassemias occur in Mediterranean populations, the Middle East, parts of India and Pakistan, Southeast Asia, and Africa. However, thalassemias have also been observed in the homozygous state in persons of pure Anglo-Saxon ancestry, so ethnic origin does not preclude the diagnosis.

In beta thalassemia, beta chain production is reduced along with a compensatory increase in gamma and delta chain production. Over 120 beta thalassemia genes have been identified which accounts for the great diversity in the phenotypic expression of this disorder. β₀ refers to a gene which produces no beta chains. β⁺ refers to a gene which produces less than normal amounts of beta chain. Production can vary from 80% to 90% of normal to less than 10%. In the homozygous condition (β₀/β₀, β₀/β⁺, or β⁺/β⁺) anemia can be moderate, requiring minimal medical intervention, to severe, requiring regular transfusions. The majority of hemoglobin present is F. Hemoglobin A is decreased or absent and hemoglobin A2 levels are slightly increased. Excess unpaired α-chains precipitate resulting in premature erythrocyte destruction and ineffective erythropoiesis. In the heterozygous condition (β⁺/β or β⁺/β) anemia can vary from slight to moderate with slight increases in A2 and F levels.

In alpha thalassemia, alpha chain production is reduced or absent. Because there are two alpha genes (identified as α₁ and α₂) on each chromosome 16, an individual may inherit one, two, three, or four defective alpha genes. α₀ refers to a deletion of both α genes on chromosome 16 (–); α– refers to a deletion of one α gene on chromosome 16 (α–). Genotypes of α-thalassemia and the resulting phenotypic expressions are shown in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Ferritin</th>
<th>Serum iron</th>
<th>TIBC*</th>
<th>% saturation</th>
<th>RDW†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>N² to ↑</td>
<td>↓</td>
<td>N to ↓</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td>↑</td>
<td>↑</td>
<td>N to ↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Thalassemia trait</td>
<td>N to ↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>This case</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

* TIBC=total iron binding capacity
† RDW-red blood cell distribution width
‡ N = normal
In α-thalassemia, excess unpaired γ and β-chains combine to form tetramers of γ (Hb Barts) and β, (Hb H). These tetramers have an extremely high oxygen affinity and are therefore poor oxygen carriers. Hb Barts and Hb H are fast moving hemoglobins and migrate past hemoglobin A on cellulose acetate at alkaline pH. Hb Barts will be present at birth but will be replaced by Hb H as the switch from γ-chain to β-chain synthesis occurs. In the silent carrier and thalassemia minor conditions, hemoglobin electrophoresis in the adult is normal. In hemoglobin H disease, Hb H levels vary from 5% to 40%. Hb H is somewhat unstable and can be induced to precipitate in the red cells by incubation of the blood with brilliant cresyl blue. Although demonstration of hemoglobin H inclusions is considered diagnostic of α-thalassemia trait, patients with α/-αα and α/-α- genotypes usually have negative results. Patients with the --/αα (trans) genotype are much more likely to test positive for hemoglobin H inclusions. Because hemoglobin electrophoresis is normal in α-thalassemia trait and the absence of Hb H inclusions does not rule out this disorder, diagnosis is problematic. These patients are at risk of producing homozygous (Barts hydrops fetalis) or doubly heterozygous offspring (Hb H disease) and therefore, must be identified for purposes of genetic counseling. The use of red cell parameters in differentiating between the cis and trans type has been reported. In one study, the --/αα genotype was associated with lower MCVs, higher RBC counts and the presence of Hb H inclusions.

In this case, the blood tested negative for hemoglobin H inclusions. The moderately decreased MCV, slightly elevated red cell count and negative test for Hb H inclusions were suggestive of either a α/-αα (silent carrier) or α/-α- (trans) genotype. The father was deceased and a CBC on the mother and only sibling showed normal results.

DNA ANALYSIS
Genotyping, while expensive and time consuming, provides the definitive diagnosis. Southern blot analysis using the restriction endonucleases Bam HI and Hind III in addition to PCR assays were performed. No α2-globin genes were detected by PCR, and Southern blot analysis confirmed the absence of the α2-globin genes on both chromosomes. These results establish the diagnosis of α-thalassemia minor with a trans type genotype of α/-α-.

This form of α-thalassemia occurs with a high frequency throughout West Africa, the Mediterranean, the Middle East, and Southeast Asia.

SUMMARY
This is a case of hypochromic, microcytic red cells in a young adult Caucasian female. It illustrates the importance of performing iron studies to confirm suspected iron deficiency anemia (IDA). Thalassemia minor is often misdiagnosed as IDA and iron therapy may be needlessly administered. Moreover, the patient will be unaware of an inherited hematological disorder which may require genetic counseling. α-thalassemia patients with the --/αα (cis) genotype should be advised of the risk for producing offspring with Hemoglobin H disease (genotype --/αα).

In this case, DNA analysis confirmed the diagnosis of a trans type gene deletion α-thalassemia trait. Ancestry on the maternal side is German and French. On the paternal side the ancestry is Dutch and Scandinavian. Additionally, there was no knowledge of any family history of anemia on either the maternal or paternal side of the family. This case reaffirms that Anglo-Saxon ancestry does not preclude the diagnosis of α-thalassemia. It also supports the findings of Wang that when laboratory findings are suggestive of α-thalassemia minor, a moderately decreased MCV, slightly elevated red cell count, and the absence of hemoglobin H inclusions is probably indicative of trans rather than cis type gene deletion α-thalassemia trait.

Table 3. α-thalassemia genotypes and phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Disorder</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>--/--</td>
<td>Hb Barts</td>
<td>Severe – fatal</td>
</tr>
<tr>
<td>--/--</td>
<td>hydrops fetalis</td>
<td></td>
</tr>
<tr>
<td>--/α-</td>
<td>Hb H disease</td>
<td>Moderate</td>
</tr>
<tr>
<td>--/αα (cis)</td>
<td>Thalassemia minor</td>
<td>Mild or none</td>
</tr>
<tr>
<td>or α-α- (trans)</td>
<td>Thalassemia</td>
<td>Minor/ Trait</td>
</tr>
<tr>
<td>α-αα</td>
<td>Silent carrier</td>
<td>None</td>
</tr>
</tbody>
</table>

REFERENCES
Career Alternative: Clinical Trials

PETER M COLANINNO

ABBREVIATIONS: CAP = College of American Pathologists; FDA = Food and Drug Administration.

INDEX TERMS: clinical trials; employment.

Clin Lab Sci 2003;16(2):82

Peter M Colaninno MS CLS(NCA) is Laboratory Manager, Infectious Diseases, Icon Laboratories Inc, Farmingdale NY, and Adjunct Professor, Allied Health Professions, at St John’s University, Jamaica NY, City University of NY, Queensborough Campus, State University of NY, Farmingdale Campus, and Hofstra University, Hempstead NY.

Address for correspondence: Peter M Colaninno MS CLS(NCA), Laboratory Manager, Infectious Diseases, Icon Laboratories Inc, 260 Smith Street, Farmingdale NY 11735.

As an adjunct professor instructing clinical laboratory science (CLS) students, I’m frequently asked about employment opportunities in the field. Historically, the proclivity of graduating students is to find employment in a hospital setting, but with hospitals under severe fiscal constraint, especially in the New York City area, they are no longer the hiring centers for clinical laboratory scientists (CLSs) that they were in the past.1,2 Because of this, graduates are forced to find career alternatives in order to utilize their degree.

There are a number of employment opportunities available to CLSs.3,4 These include:

Technical: Private laboratories; physician’s office laboratories; college research laboratories; health maintenance organization and union health offices; government laboratories; state and city health departments; blood banks; veterinary offices.

Non-technical: Inspector; infection control officer; quality assurance officer; safety officer; laboratory management; planning and development; information technology; risk management officer; consultant.

Education: Teaching; administration; mentoring.

Commercial: Marketing; sales; public relations; technical writing and illustrations.5

However, a career alternative that has come to the forefront in recent years is employment in a clinical trials laboratory.

What are clinical trials?
Clinical trials laboratories work closely with pharmaceutical companies to assist them in the FDA approval process of new compounds and are dedicated to testing samples procured from patients who are enrolled in phase 2, phase 3, and phase 4 trials, testing the efficacy of compounds being introduced by the pharmaceutical companies. After working in a hospital setting for more than 17 years as a microbiologist, I made the transition five years ago to a clinical trials laboratory and quickly found out that these companies are major employers of CLSs, including graduates with an associate degree as well as a baccalaureate degree (Table 1).

How does the work differ from that of a clinical laboratory?
Clinical trials laboratories perform laboratory testing much

| Table 1. Employment opportunities in clinical trials |
|-------------|-------------|
| Associate degree | Bachelor degree |
| Laboratory | X | X |
| Project management | X |
| Client service | X | X |
| Quality assurance | X |
| Information technology | X |
| Safety officer | X |
| Business development executive | X |
| Educational coordinator | X |
CLINICAL PRACTICE: IN PRACTICE

like a clinical laboratory but, because of the proprietary nature of the business, there is a high degree of confidentiality. CLSs perform not only basic laboratory testing, but specialized testing as well. A typical clinical trials laboratory includes: chemistry; hematology; coagulation; urinalysis; toxicology; microbiology; immunology; molecular biology; and cellular immunology. Work in this environment is rewarding and offers unique challenges. For example, a clinical microbiology laboratory may have a limited number of antibiotic susceptibility panels to be used on specific pathogens, i.e., gram positives, gram negatives, *Haemophilus sp.* However, in clinical trials, not only are there different antibiotic susceptibility panels for each pharmaceutical company’s compound and comparator drug, there may be different panels needed depending on the anatomical site from which a specimen was procured. Jennifer Welsch, supervisor of Infectious Diseases at Icon Laboratories agrees, “With so many different studies ongoing, it is imperative to develop systems that will ensure that the correct antimicrobial panel will be used for the proper study. In a hospital setting, there are set formularies for gram positives, gram negatives, and urine isolates. Clinical trials offer a unique challenge with studies from a multitude of pharmaceutical companies, each with their own compound and comparator drug.”

What are some other areas of employment available to CLSs? There are many other employment opportunities within a clinical trials company. These include:

**Project management:** Project management is another department that employs CLSs. This department follows each protocol awarded to the company from the inception of the study until its completion. A project manager is assigned to each protocol and is responsible for all facets of the protocol from its setup to: working with clinical research associates, sponsors, investigators, data personnel, and study coordinators; reviewing management reports, patient status reports, and analyte trend reports; and examining logs of cancelled tests and abnormal result reports. The project manager is the point person for the study and must have knowledge of all of the tests that are included in the study. A background in CLS is essential in order to troubleshoot any problems that may arise during the study in regard to the laboratory testing. This is an administrative position that many former bench technologists covet as they continue their career in CLS. Terrie Mannix, associate director of project management at Icon Laboratories states, “After working for a number of years as a bench technologist, I was looking for alternative careers in which I could utilize my degree. My position at Icon allows me to use my laboratory experience in setting up protocols, as these protocols have a wide diversity of laboratory tests associated with them.”

**Client service:** Client service personnel act as liaisons between the investigator sites that collect the clinical samples and the laboratory. Their duties include: informing sites on the proper sample to procure for the study; managing supplies for the investigator sites; tracking packages of supplies as well as samples; providing information on the proper packaging of samples; examining turnaround time and stability of samples; amending any necessary patient demographics; and faxing selected reports to the investigators. Client service employs both baccalaureate and associate degree CLSs.

**Quality assurance:** Pharmaceutical companies routinely conduct audits of the laboratory to ensure quality assurance exists for their protocols. The quality assurance department prepares for the audits conducted by the pharmaceutical companies and is the lead department for inspections by regulatory agencies such as the FDA and CAP as well. Additionally, the quality assurance department, to ensure that the work being performed is of the highest quality, conducts regular internal audits of the laboratory. The quality assurance department reviews all competency records, standard-operating protocols, training records, and validations.

**Information technology:** Information technology departments offer employment to CLSs who are proficient with computers and programming. Formulation and creation of laboratory worksheets, mnemonics, Levy-Jennings charts, test panels, reference ranges, quality control panels, and instrument interfacing are areas in which a laboratory background is helpful. Customarily, a four-year degree is required.

**Safety officer:** The safety officer is the individual responsible for the overall safety of the facility. Duties of the safety officer include: performing safety audits, safety lectures, and safety monitors; conducting fire drills; reviewing incident reports and safety related issues; writing the safety standard operating procedure manual; ordering personal protective equipment; establishing a chemical hygiene plan; formulating a waste management program; and coordinating safety meetings. Because the safety officer must be familiar with the laboratory and its various tests, a CLS is qualified for this position.

**Business development:** Business development executives are responsible for preparing presentations to the various phar-
maceutical companies that detail the spectrum of testing the laboratory offers. They serve as liaisons between clients and the laboratory and help clients reach their goals by generating ideas and solving problems. Additionally, business development executives update clients on trends and market conditions; serve as information resources; provide sales and customer service training; and organize educational support for clients. They interpret a client’s requirements and match the client’s needs with the laboratory’s capabilities for a successful trial. Obviously, knowledge of the laboratory and its testing capabilities is of paramount importance for business development executives. CLSs with a baccalaureate degree are eligible to serve in this capacity.

Educational coordinator: Some clinical trials companies offer internships for students enrolled in clinical laboratory courses. The laboratory may offer these internships either alone or through an affiliation with a local university. The educational coordinator must organize clinical rotations for students; provide educational material where needed; formulate examinations and unknowns; and perform didactic and technical training. The coordinator is responsible for the training and safety of students in the facility. CLSs with a four-year degree and experience in teaching can fill the position of educational coordinator.

What are some advantages and disadvantages of working in clinical trials?
As you can see, clinical trials laboratories offer a number of various career options to the CLS. They offer the unique opportunity for an individual to utilize his/her degree in a number of different areas, all within the same company. However, because these laboratories are not located in primary or tertiary healthcare facilities, some areas of CLS such as blood bank and phlebotomy are not germane to these facilities.

Do clinical trials laboratories offer competitive salaries and benefits?
Many of these companies offer competitive salaries, yearly increases, and performance bonuses; full benefits, such as medical, dental, vision and life-insurance; retirement plans and profit sharing; the availability of different shifts; and advancement up the professional ladder as well as other perquisites, such as night differential and tuition reimbursement.

How does one find out about clinical trials laboratories?
Laboratories that deal solely in clinical trials include Icon, Covance, Quintiles, and Barc, while others such as LabCorp and Quest handle not only clinical trials samples but clinical samples as well. Many of these companies advertise in national publications such as Advance; their Web addresses are listed in Table 2.

If you are looking for a career alternative, it would certainly be to your advantage to include clinical trials on your list as a rewarding and satisfying career.

REFERENCES
2. Personal Communication 2002. Advisory Committee Meeting, Farmingdale State University, Farmingdale NY.

Table 2. Clinical trials laboratories contact information

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Web Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barc</td>
<td><a href="http://www.za.barclab.com">www.za.barclab.com</a></td>
</tr>
<tr>
<td>Covance</td>
<td><a href="http://www.covance.com">www.covance.com</a></td>
</tr>
<tr>
<td>Icon Laboratories</td>
<td><a href="http://www.iconlabinc.com">www.iconlabinc.com</a></td>
</tr>
<tr>
<td>LabCorp</td>
<td><a href="http://www.labcorp.com">www.labcorp.com</a></td>
</tr>
<tr>
<td>Quest</td>
<td><a href="http://www.questdiagnostics.com">www.questdiagnostics.com</a></td>
</tr>
<tr>
<td>Quintiles</td>
<td><a href="http://www.quintiles.com">www.quintiles.com</a></td>
</tr>
</tbody>
</table>
HIPAA Privacy Rule: The Debate Continues

KARRIE B HOVIS, DIANNA M VEILLON

ABBREVIATIONS: DHHS = Department of Health and Human Services; HIPAA = Health Insurance Portability and Accountability Act; LIS = laboratory information system; PHI = protected health information; POL = physician office laboratory.

INDEX TERMS: HIPAA.

Clin Lab Sci 2003;16(2):85

Karrie B Hovis CLS(NCA), is an Instructor, Dianna M Veillon MD is an Associate Professor, Louisiana State University Health Sciences Center, Shreveport LA.

Address for correspondence: Karrie B Hovis CLS(NCA), Louisiana State University Health Sciences Center, 1107 Mountainbrook Dr, Shreveport LA 71118. (318) 675-6807, (318) 675-6937 (fax). Khovis@lsuhsc.edu

The Fourth Amendment of the United States Constitution guarantees that “the right of the people to be secure in their persons, houses, papers, and effects against unreasonable searches and seizures, shall not be violated.” In other words, the Fourth Amendment guarantees the privacy of Americans. Yet, many Americans feel that this freedom has been violated when discussing the privacy of their medical information. They are concerned that the privacy of their medical information is not protected. In January 1999, a national survey conducted by the California HealthCare Foundation found that one in five Americans feel that their health information is being disclosed inappropriately. Without this trust, patients are compromising their own healthcare either by providing inaccurate information to their physician, changing physicians, or avoiding care altogether.1 With the rising concern about patient privacy, the United States federal government realized that something needed to be done.

On August 21, 1996, the Health Insurance Portability and Accountability Act (HIPAA) was enacted. Although this legislation was well intended, its supporters failed to recognize several potential problems. One of the biggest shortfalls was the fact that patient health information may be exposed without patient consent. Even though all states had laws in effect to cover this deficit, the laws varied from state to state. The Department of Health and Human Services (DHHS) has subsequently issued another law entitled the HIPAA Privacy Rule. This law is expected to prevent exposure of a patient’s confidential medical information. New questions have arisen. Should the HIPAA Privacy Rule override current state legislation? Has the federal government overstepped its boundaries this time?

When HIPAA was first enacted, its primary purpose was to ensure that all workers who lost or changed jobs were able to maintain health insurance. The law, however, included significant changes concerning fraud and abuse in healthcare and encouraged the establishment of medical savings accounts. HIPAA also attempted to simplify the administration of health insurance by encouraging electronic transmission of certain transactions.2 By allowing these electronic transactions to occur, the government was expected to save $29.9 billion over ten years.3 In all the calculations of potential savings, however, the cost of regulation was not included. By allowing electronic transactions to occur, the federal government introduced another problem: patient privacy. With concerns of patients’ protected health information (PHI) being exposed, the government had to issue another set of regulations to cover this deficiency. This set of regulations is included under the HIPAA Privacy Rule.

Several groups are affected by the implementation of the HIPAA Privacy Rule. Any organization that transmits patient health information electronically is considered a covered entity under the HIPAA Privacy Rule. These organizations include health plans, healthcare clearinghouses, and healthcare providers. Most group health plans, health insurance carriers, health maintenance organizations (HMOs), and federal health programs are included. Therefore, if you are a recipient of Medicare benefits, the Privacy Rule will protect your PHI.4
CLINICAL PRACTICE

Clearinghouses are organizations that are considered the ‘middle man’ of insurance claims. They are responsible for translating data received from the payee to the payor. In other words, an insurance claim that is received electronically from a hospital undergoes data translation before the final bill is sent to the patient. The healthcare clearinghouse is responsible for the interpretation and the billing process. Controversially, some clearinghouses are selling their gathered information to the private sector. For example, pharmaceutical companies frequently purchase information from these clearinghouses for research purposes and market analysis.4

Healthcare providers include physician offices, pharmacies, and hospitals. A healthcare provider is defined as “a provider of healthcare, medical, or health services as defined in the Act (HIPAA), or any other person or organization that furnishes, bills for, or is paid for healthcare services or supplies in the normal course of business”4.

According to the Privacy Rule, a covered entity must make “all reasonable effort” not to disclose patient information that will not be used for the intended purpose. This requirement is referred to as the “minimum necessary” rule. Under this rule, a covered entity must be careful about which patient information is made known, but this has the potential to jeopardize patient care.5 Although a trustworthy physician/patient relationship is essential, limiting the use of information while trying to provide adequate services can potentially present a problem.

If a covered entity must disclose patient information to an outside source who is a business associate, a confidentiality contract must be reached beforehand. Not only does this create more paperwork, but also the covered entity is held liable for breach of patient information if the contract is not upheld. Although the covered entity need not actively monitor the outside source, they must ensure that all business associates adhere to the original contract. It is the responsibility of the covered entity to adopt written privacy procedures to address this issue and to investigate credible evidence of contract violation.3

The HIPAA Privacy Rule also introduced the term “privacy officer”. Each covered entity must appoint someone to fulfill this position. The purpose of this individual is to ensure that the entity’s privacy procedures are followed.3 In many cases, this responsibility will be delegated to an individual who already has several compliance duties. On October 22, 2001, two congressmen, Representatives John Peterson (R-PA) and John Murtha (D-PA), urged Congress to work with the DHHS to reduce the burden that HIPAA is placing on the healthcare community. They wanted “to see that the final (HIPAA privacy) regulations do not get in the way of the heroic work that hospitals do every day.”6

One advantage to the HIPAA Privacy Rule is that individuals will be able to inspect, copy, and request changes to their medical records. Many state privacy laws currently address this issue as well. Under the HIPAA regulations, the covered entity will be allowed to charge a fee to offset copying expenses. This could create a hardship for the poor.3

Another problem with individuals inspecting their medical records is the refusal of the covered entities to make any changes to the records. Covered entities will need to devise guidelines for changing or refusing to change medical records. For example, a patient might request a change in the date of a service if the service predated his/her insurance coverage.4 Although the covered entity is correct in refusing the change, this could precipitate a conflict.

Where does the clinical laboratory fit into the picture of patient privacy? We are professionals who provide a service for the patient, and we bill for that service. Therefore, this classifies the laboratory as a healthcare provider. However, there are two types of healthcare providers: direct and indirect. The most part, clinical laboratories are going to be considered indirect healthcare providers. Most clinical laboratories, such as hospital laboratories and reference laboratories, have an indirect relationship with the patient.7 As laboratorians, we interact with the physician, not the patient. The Privacy Rule does not require clinical laboratories to provide patient access to laboratory results since the Clinical Laboratory Improvements Amendments of 1988 (CLIA ’88) prohibits this service. Patients can only inspect and copy their laboratory results through the healthcare provider. The only exception to this rule is if state law permits patients to gain access to laboratory results. If the state law defines an “authorized person” to include the patient, then the patient can gain access of their laboratory results through the laboratory personnel.8

POLs are in a direct relationship with the healthcare provider. Due to their location, these laboratory personnel offer their services directly to the patient. Therefore, it is imperative that these facilities have policies and procedures in place to address the concern of patient privacy.
As for research laboratories, the privacy regulations still apply but have different applications. For instance, some laboratory results can be released directly to the patient in a research setting. If the laboratory result will not be used for purposes of diagnosis or treatment, then the patient can receive the laboratory result without having to go through the ordering individual.8

Should the laboratory offer a separate consent form? According to the HIPAA Privacy Rule, a separate consent form is not needed. We perform a service for the patient, but the doctor requests the service. The argument has been made that in states where the state law permits the patient to gain access directly to the laboratory results then a consent form (or some other legal document) should be necessary.9 Numerous instances have been reported in which laboratory professionals released results directly to the patient. In some cases, a consent form was signed, but in most, there was no documentation made. Before any results are released directly to the patient, laboratory professionals should have knowledge about what their state law allows.

Other practices, such as the reporting of communicable diseases to public health authorities, are excluded from the Privacy Rule.8 It is the responsibility of clinical laboratory personnel to report such findings as methicillin-resistant Staphylococcus aureus to the public health officials. Such findings are required by law for the purposes of public health and will continue to be reported without a confidentiality contract.

As for significant changes that will directly affect all laboratory personnel, there will be few. The HIPAA Privacy Rule will now regulate all current and historical data stored in an institution’s laboratory information system (LIS). Changes in transaction codes that will be implemented by the LIS vendor may require some computer downtime.

Some LIS vendors require facilities to release protected health information in order to troubleshoot problems. This practice will become more limited with the new privacy regulations. Also, more stringent rules will be put in place for employees to gain access to facility-wide systems. This access will be granted on a ‘need to know’ basis. All healthcare professionals will be required to undergo some form of privacy training at their facility as part of compliance. Some facilities may require employees to document and keep copies of all facsimile transmission reports. This is to ensure that the right person received the report. Fax numbers may also have to be verified before transmission.10

Currently, there is a meshwork of state laws that address this issue. While several states have laws that enforce very strict confidentiality regulations, some states have laws that are more lenient. Thus, there is no uniformity involving the protection of a patient’s PHI. The HIPAA Privacy Rule is the federal government’s first attempt to govern the privacy of PHI. According to the DHHS, this rule will provide the groundwork for regulation of a patient’s health information.5

Some state laws conflict with the Privacy Rule and also with each other. The Privacy Rule will supersede any state laws that are not sufficiently strict. A major concern is that certain areas of the country have situations that are not conducive to the federal law. Federal legislation does not address a population’s specific needs. On the other hand, insurance companies, who frequently operate across state lines, are very much in favor of federal regulations. They claim that allowing federal regulations to override state laws will result in decreased costs to the consumer.7

Most Americans agree that we are in desperate need of more strict confidentiality regulations. Before the Privacy Rule was issued, our employers could gain access to our health records. A patient’s entire medical record could be released to an employer even if only a portion of the information was requested.3 In the past, this practice has affected hiring and promotion. However, with the new privacy law in place, people are now scared that the government will have more access to their health records.11 The Office for Civil Rights (OCR) can access an individual’s PHI without his/her consent if there is reason to suspect that the Privacy Rule has been violated.12 The Food and Drug Administration (FDA) can also access patient information in effort to regulate “the quality, safety, or effectiveness of FDA-regulated products or activities.”13

On April 14, 2003, the nation’s healthcare system will have to be in compliance with the HIPAA Privacy Rule. Many organizations will have difficulty meeting this deadline. For some, the problem will be lack of preparation.11 For other organizations, the problem may be a more personal decision to prevent more governmental control over our nation’s healthcare system. The Privacy Rule is a popular item of debate, and both sides of the debate are condemnatory of the rules.5 Whatever the outcome, the delivery of healthcare will be different, and the change will be felt by many.
REFERENCES

# ANNUAL MEETING 2003 PROGRAM

## TUESDAY

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
<th>Governance</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 am – 10:00 am</td>
<td>Clinical Lab Expo (9:30 am– 5:00 pm)</td>
<td></td>
<td>ASCLS Board of Directors Meeting</td>
</tr>
<tr>
<td>10:00 am – 10:30 am</td>
<td>Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30 am– 12:00 pm</td>
<td>Clinical Lab Expo (9:30 am– 5:00 pm)</td>
<td></td>
<td>ASCLS Board of Directors Meeting (continued)</td>
</tr>
<tr>
<td>12:00 pm– 1:00 pm</td>
<td>Lunch Break</td>
<td></td>
<td>Committee Chairs Orientation</td>
</tr>
<tr>
<td>1:00 pm – 2:30 pm</td>
<td>Clinical Lab Expo (9:30 am– 5:00 pm)</td>
<td></td>
<td>Moderator Orientation</td>
</tr>
<tr>
<td>2:30 pm – 2:45 pm</td>
<td>Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:45 pm – 4:15 pm</td>
<td>Clinical Lab Expo (9:30 am– 5:00 pm)</td>
<td></td>
<td>Continuing Education Advisory Council (CEAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Political Action Committee (PAC) Board</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P.A.C.E. Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CLS Consulting Editors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Presidents’ Seminar</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Professional Affairs Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Student Forum Orientation</td>
</tr>
<tr>
<td>4:15 pm – 4:30 pm</td>
<td>Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:30 pm – 6:00 pm</td>
<td>Clinical Lab Expo (9:30 am– 5:00 pm)</td>
<td></td>
<td>Government Affairs Committee (GAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Scientific Assembly Chairs (SA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Educational Affairs Committee (CEA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Awards Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Abstract Review Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Publications Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nominations Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Judicial Committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bylaws Committee</td>
</tr>
<tr>
<td>6:00 pm – 7:30 pm</td>
<td>NCA/NAACLS Update</td>
<td></td>
<td>Alpha Mu Tau Board</td>
</tr>
<tr>
<td>7:00 pm – 8:00 pm</td>
<td>First Timers Reception (All first time professional and student registrants invited)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:00 pm – 9:30 pm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## THURSDAY

**Thurs., July 24, 2003**

### 8:00 am – 8:30 am
**Shuttles to Convention Center**

### 8:30 am – 10:00 am
- **#15** Meeting the Requirements of Training and Competency in Today's Clinical Microbiology Laboratory MIC, ADM
- **#16** Hospital and Blood Center Communications: Can We Talk!!? I/H
- **#17** Performing the Appropriate Coagulation Assays HEM
- **#18** Leadership Development Seminar PSDT
- **#19** The Laboratory's Role in Outcomes: A Physician's Perspective Joint Plenary with AACC GEN, ADM

### 10:00 am – 1:30 pm
**Dedicated Exhibit Hours** *(Exhibits Open 9:30 am– 5:00pm)*

### 12:00 pm – 1:00 pm
**Administrators/Industry/Consultants Lunch**
**Men’s Lunch**

### 1:30 pm – 3:00 pm
- **#20** Prion Diseases MIC, IH
- **#21** Developing, Maintaining, and Retaining a Competent Laboratory Team EDU, ADM, GEN
- **#22** Roadmap to Minimize Laboratory & Medical Errors ADM, GEN
- **#23** Lipoproteins – From Basic Research to Clinical Application BUL

### 3:00 pm – 3:15 pm
**Break**

### 3:15 pm – 4:45 pm
- **#24** Vancomycin Resistance in Staphylococci MIC
- **#25** Blood Bank Case Studies I/H
- **#26** Sickle Cell Disease: Diagnosis and Current Management HEM
- **#27** Let's Start a Membership Epidemic PSDT

### 4:45 pm – 5:15 pm
**Shuttles to Hotel**

### 5:15 pm – 6:30 pm

### 6:30 pm – 9:00 pm
**'nT Boot Scootin' Boogie Bash** *(All registrants invited)*

---

**Governance**

- 2004 CLEC Planning Meeting *(10:00 am – 1:30 pm)*
- "Meet the Candidates" *(10:30 am – 12:00 pm)*
- President-Elect Seminar *(1:30 pm – 4:45 pm)*

---

**Scientific Assembly Meetings:**
- Education
- Laboratory Administration
- Inspectors/Surveyors
- Consultants
- Industry

- Student Forum Elections *(5:30 pm– 6:30 pm)*
### FRIDAY

**Fri., July 25, 2003**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:30 am – 8:45 am</td>
<td>Break</td>
</tr>
<tr>
<td>8:45 am – 9:00 am</td>
<td>Break</td>
</tr>
<tr>
<td>9:00 am – 10:30 am</td>
<td>#28 Reducing the Burden of Disease — Laboratory Information Supports Medical Risk and Quality Management GEN, ADM</td>
</tr>
<tr>
<td></td>
<td>#29 TechPrep Curriculum: Image-Builder and Recruitment Tool for the CLS Profession EDU</td>
</tr>
<tr>
<td></td>
<td>#30 West Nile Virus — Addressing the Risk to the U.S. Blood Supply Through Nucleic Acid Testing MIC, IH, GEN</td>
</tr>
<tr>
<td></td>
<td>#31 Member Submitted Case Studies GEN</td>
</tr>
<tr>
<td></td>
<td>W-1 Update: Laboratory Coding, Billing, Reimbursement and Compliance ADM, GEN</td>
</tr>
<tr>
<td></td>
<td>W-2 Body Fluid Cell Counts and Morphology: A Pediatric Perspective HEM</td>
</tr>
<tr>
<td>10:30 am – 10:45 am</td>
<td>Break</td>
</tr>
<tr>
<td>10:45 am – 12:15 pm</td>
<td>#32 Proteomics Analysis of Malignant Lymphomas GEN, BUL, HEM</td>
</tr>
<tr>
<td></td>
<td>#33 Transfusion-related Acute Lung Injury I/IH</td>
</tr>
<tr>
<td></td>
<td>#34 Clinical Practice Model GEN, EDU, ADM</td>
</tr>
<tr>
<td></td>
<td>#35 Writing for Publication GEN</td>
</tr>
<tr>
<td></td>
<td>W-1 Continued</td>
</tr>
<tr>
<td></td>
<td>W-2 Continued</td>
</tr>
<tr>
<td>12:15 pm – 12:30 pm</td>
<td>Break</td>
</tr>
<tr>
<td>12:30 pm – 2:15 pm</td>
<td>Break</td>
</tr>
<tr>
<td>2:15 pm – 3:45 pm</td>
<td>#36 Hematology — Magnificent Morphology: Melding Mechanisms and Molecules HEM</td>
</tr>
<tr>
<td></td>
<td>#37 proBNP and the Patient with Heart Failure BUL, GEN</td>
</tr>
<tr>
<td></td>
<td>#38 Gram Stain Update: Improving Proficiency in Diagnostic Interpretation and Reporting MIC</td>
</tr>
<tr>
<td></td>
<td>#39 Member Submitted Research Papers GEN</td>
</tr>
<tr>
<td></td>
<td>W-3 Are Your Students Team Players? EDU</td>
</tr>
<tr>
<td></td>
<td>W-4 Laboratory Ergonomics Awareness: Fitting the Workplace to the Worker GEN, ADM</td>
</tr>
<tr>
<td>3:45 pm – 4:00 pm</td>
<td>Break</td>
</tr>
<tr>
<td>4:00 pm – 5:30 pm</td>
<td>#40 Meeting Planning PSDT</td>
</tr>
<tr>
<td></td>
<td>#41 Urine Microscopic Examinations: Challenges and Clinical Correlations BUL, HEM</td>
</tr>
<tr>
<td></td>
<td>#42 Agents of Bioterrorism: Beyond Anthrax and Smallpox — Recognition and Laboratory Identification MIC, GEN</td>
</tr>
<tr>
<td></td>
<td>#43 Member Submitted Research Papers/Student Papers GEN</td>
</tr>
<tr>
<td></td>
<td>W-3 Continued</td>
</tr>
<tr>
<td></td>
<td>W-4 Continued</td>
</tr>
<tr>
<td>Time</td>
<td>Event/Activity</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>8:30 am – 9:30 am</td>
<td>#44 Closing Keynote: Reflections</td>
</tr>
<tr>
<td>9:30 am – 10:00 am</td>
<td>Break</td>
</tr>
<tr>
<td>10:00 am – 12:30 pm</td>
<td>Lunch Break (On Own)</td>
</tr>
<tr>
<td>12:30 pm – 2:00 pm</td>
<td>Minority Forum Luncheon (Ticketed)</td>
</tr>
<tr>
<td>2:00 pm – 3:30 pm</td>
<td>Past Presidents’ Luncheon (Invitation Only)</td>
</tr>
<tr>
<td>3:30 pm – 4:00 pm</td>
<td>Break</td>
</tr>
<tr>
<td>4:00 pm – 5:00 pm</td>
<td>President’s Reception (Invitation Only)</td>
</tr>
</tbody>
</table>

* = Requires pre-registration and additional fee ($); see page 29.
**REPORTS AND REVIEWS**

**Method Comparison Studies for Prostate Specific Antigen and Unconjugated Estriol Immunoassays**

MARY E KOENN, BONIFACE V NDAH

OBJECTIVE: Method comparison studies were performed in order to move a semi-automated prostate specific antigen (PSA) immunoassay and a manual unconjugated estriol (uE3) immunoassay to an automated chemistry immunoassay analyzer. The results of the two method comparison studies are compared.

DESIGN: Serum samples collected on patients with physician orders for PSA or uE3 were assayed by both methods. PSA samples were assayed on a Hybritech Tandem Photon ERA and on two Beckman Coulter Access instruments. Urine samples were assayed by RIA and on two Beckman Coulter Access instruments. Linear regression analysis was performed on both sets of data and within-run precision and dilution studies were performed on the PSA Access method.

SETTING: Clinical chemistry laboratory, West Virginia University Hospitals Inc, Morgantown WV.

RESULTS: PSA linear regression analysis for the two methods (ERA and Access 1) were $y = 1.0008x + 0.0393$, $r = 0.9976$, SE = 0.1319, n = 37 and (ERA and Access 2), $y = 1.0019x + 0.0486$, $r = 0.9964$, SE = 0.1632, n = 37. Within-run precision studies for both Access instruments produced acceptable coefficient variations and dilution study results were in PSA reportable range. uE3 linear regression analysis for the two methods (RIA and Access 1) were $y = 1.4105x - 0.3741$, $r = 0.8696$, SE = 0.8330, n = 33 and (RIA and Access 2) were $y = 1.315x - 0.2292$, $r = 0.8643$, SE = 0.7964, n = 33.

CONCLUSION: The results of the method comparison studies for PSA were acceptable and the automated PSA immunoassay method was adopted. The results of the uE3 comparison studies did not show good correlation; the automated method was not adopted.

ABBREVIATIONS: CV = coefficient of variation; MoM = multiple of the median; PSA = prostate specific antigen; r = correlation coefficient; RIA = radioimmunoassay; SE = standard error; uE3 = unconjugated estriol.

INDEX TERMS: immunoassay; method comparison studies; prostate specific assay; unconjugated estriol.

Clin Lab Sci 2003;16(2):94

Mary E Koenn MS CLS(NCA) is in the Medical Technology Program, West Virginia University, Morgantown WV.

Boniface V Ndah MD is in the Department of Pathology, West Virginia University, Morgantown WV.

Address for correspondence: Mary Ellen Koenn MS, Medical Technology Program, West Virginia University, Room 2163C, PO Box 9211, Morgantown WV 26506-9211. (304) 293-1632, (304) 293-6249 (fax). MKoenn@hsc.wvu.edu

Among American men, prostate cancer is the most common malignancy and the second leading cause of cancer mortality. According to autopsy studies, approximately nine million American men may now have prostate cancer.1 In most patients, the malignancy grows slowly, resulting in different grades of tumor confined to the prostate gland and at this stage, often still curable. Rapid growth and metastasis beyond the prostate are seen in some patients creating a less favorable long-term survival. Early detection along with local treatment is necessary in the management of this disease.2

Although controversial, prostate specific antigen (PSA) determination, in conjunction with digital rectal examination (DRE) are currently recommended for screening all men age 50 and older.1 Other uses of PSA levels include pretreatment staging and post-treatment monitoring.2,5 Screening for prostate cancer with PSA has significantly increased the volume of PSA tests performed by clinical laboratories. A marked increase in reported incidence of prostate cancer has also occurred. Since PSA is found in benign prostatic hyperplasia and inflammatory conditions of the prostate, interpretation of results should be used with DRE or ultrasound.5

UNCONJUGATED ESTRIOL

Estrogens are primarily secreted by the ovarian follicles and the corpus luteum in the non-pregnant females; in pregnancy, the major source is the placenta. The primary estrogen secreted by the ovary is estradiol, whereas that of the placenta is estriol. The placenta forms estriol by sequential desulfurylation and aromatization of the androgen,
dehydroepiandrosterone sulfate (DHEA-S). Approximately 9% of the estriol remains unconjugated and is tightly bound to sex hormone-binding globulin. The majority is conjugated to glucuronates and sulfates in the maternal liver permitting renal clearance.

Maternal serum testing is a well-established screening procedure for the detection of congenital anomalies. Measurement of unconjugated estriol (uE₃) in the third trimester was first used to assess fetal well being.

During the second trimester, serum levels of uE₃ are decreased in fetal Down syndrome. A combination of uE₃, alpha fetoprotein (AFP) and chorionic gonadotropin (CG) as a triple marker is now used for fetal screening at 16 weeks gestation for Down syndrome. Like uE₃, AFP is decreased in Down syndrome, while CG is increased. Reporting the triple screen results in conventional units and as multiple of the median (MoM) identifies about 5% of patients with increased risk of Down syndrome and detects approximately 60% of actual cases of Down syndrome. Low levels of uE₃ are also associated with pregnancy-induced hypertension, miscarriage, intrauterine growth restriction, and intrauterine fetal death.

MATERIALS AND METHODS
The clinical chemistry laboratory performed method validation studies to move a semi-automated PSA immunoassay and a manual uE₃ immunoassay to an automated chemistry immunoassay analyzer. All study assays were performed according to manufacturers’ recommendations; calibrators, standards, and controls for assays were utilized according to laboratory approved procedures.

Semi-automated PSA
The Hybritech Tandem Photon ERA (Beckman Coulter Inc, Fullerton CA), a two-site immunometric (sandwich) semi-automated assay, quantitates PSA in serum samples. The capture monoclonal antibody is against a unique site on the PSA molecule, and the other antibody, enzyme-labeled, is directed against a different PSA molecule site. The assay is completed with the addition of the photon enzyme substrate and the color formed is measured in the PHOTON ERA instrument. The quantity of color detected is proportional to the concentration of PSA in each sample.

Manual uE₃
The Ultra-Sensitive uE₃ (Diagnostic Systems Laboratories Inc, Webster, TX) is a competitive binding manual radioimmunoassay. The antigens, 125I-labeled uE₃ and patient serum sample uE₃, compete for a fixed number of antibody (rabbit anti-uE₃) binding sites. After incubation and formation of antigen-antibody complexes, a double antibody system separates free antigen from the antibody-bound antigen. For this separation, a goat anti-rabbit precipitating reagent is added and centrifugation completes the separation. A Gamma Counter (Packard Instrument Co, Downers Grove IL) measures the amount of bound 125I-labeled uE₃; the count is inversely proportional to the concentration of uE₃ present in the patient sample.

Automated immunoassay analyzer methods
For the comparison studies, both sets of patient samples were assayed on each of two Beckman Coulter Access Immunoassay analyzers (Beckman Coulter Inc, Fullerton CA). The Access is a chemiluminescent immunoassay measurement system using paramagnetic particles coated with antibody to the analyte of interest and the chemiluminescent substrate, dioxetane phosphate. Alkaline phosphatase-labeled complexes react with the substrate creating a chemical reaction and a source of energy to excite the dioxetane substrate. The light emitted is quantitated in the analyzer luminometer.

The PSA Access assay is a two-site immunometric assay using the same Hybritech antibodies as the Photon ERA method. The anti-PSA coating the paramagnetic particles captures the PSA molecule and a second antibody labeled with alkaline phosphatase completes the sandwich by binding to a different antigenic site on the PSA molecule. Addition of the dioxetane substrate initiates the chemiluminescent reaction and the amount of light measured in the luminometer is proportional to the concentration of PSA in the sample.

The uE₃ Access assay is a competitive binding immunoenzymatic assay; serum uE₃ and alkaline phosphatase-labeled uE₃ compete for a fixed number of uE₃ antibodies. The antigen-antibody complexes attach to the paramagnetic particles and the enzyme-labeled complexes initiate the chemiluminescent reaction. The amount of light quantitated in the luminometer is inversely proportional to concentration of uE₃ in patient samples.

Split-sample comparative studies, 40 samples for PSA and 33 for uE₃ studies, were assayed with present laboratory methods and then on the Access 1 and Access 2 instruments. Method comparison studies were performed to derive the linear regression equation, correlation coefficient (r), and standard error (SE). Further statistical testing of within-run precision and linear range completed the PSA evaluation.
RESULTS
PSA
Linear regression analysis showed good agreement between the semi-automated Hybritech Photon ERA PSA and the automated Access 1 and Access 2 immunoassay measurements. Three high PSA results (outliers) were discarded. The linear regression plot and equation for ERA and Access 1 is shown in Figure 1. Analysis resulted in $r = 0.9976$, $SE = 0.1319$ for $n = 37$. See Table 1, 0-10 $\mu$g/L. Comparison analysis for the ERA and Access 2 were very similar, $r = 0.9964$, $SE = 0.1632$ for $n = 37$. See Figure 2, 0-10 $\mu$g/L and Table 2.

Within-run precision studies for both analyzers resulted in acceptable coefficient of variations (CV), Access 1, CV = 1.7 and Access 2, CV = 1.9. Dilution studies to assess the method linearity showed results within the PSA reportable range.

\[ uE_3 \]
Regression analysis for \( uE_3 \) did not display comparable agreement. Figure 3 displays the regression plot and equation for RIA and Access 1 assays, $r = 0.8696$, $SE = 0.8330$ for $n = 33$. (Table 3) Similar plots and statistics resulted with RIA and Access 2 (Figure 4), $r = 0.8643$, $SE = 0.7964$ for $n = 33$ (Table 4).

DISCUSSION
PSA
Because of the good agreement resulting from the method comparison and the discontinuance of the Hybritech reagents for the Photon ERA, the PSA testing was moved to the Access analyzers. Use of the same antibodies by both methods contributed to the good correlation.

Assaying PSA specimens on an automated analyzer provides significant advantages for the chemistry laboratory. The Access, a random-access immunoassay analyzer, reduces the hands-on labor time and is interfaced to the laboratory information system, all resulting in faster turnaround time for result reporting. The laboratory has performed all PSA tests on the Access instruments for the past 14 months and proficiency testing has been satisfactory.

The effect of the concentration range on the \( r \) and \( SE \) can be explained with the data from this PSA method study. Including all 40 sample results increases the concentration range from 0-100 $\mu$g/L. For the Access 1, this improves the \( r \) from 0.9976 to 0.9996, but also increases the \( SE \) from 0.1319.
to 0.5870. Likewise, using samples with a concentration range 0-4 µg/L, decreases the r to 0.9905, but improves the SE to 0.1264 (Table 1). See Table 2 for similar results for Access 2.

\[ \text{uE}_3 \]

The correlation coefficients resulting from the \text{uE}_3 comparisons for both instruments were less than 0.99. In an attempt to expand the concentration range, additional samples would have had to be collected; otherwise, a different kind of statistical analysis would have been needed to complete the evaluation. The laboratory decided that neither of these approaches was justifiable given the time that must be expended and its new direction. The differences in results between the two methods would cause further complications incorporating \text{uE}_3 results in the MoM. Also, at this time the laboratory began an investigation in possible adoption of a different automated immunoassay analyzer.

CONCLUSION

The analysis showed good agreement between the semi-automated Hybritech Photon ERA PSA and the automated Beckman Coulter Access 1 and Access 2 immunoassay measurements. The Beckman Coulter Access PSA method was adopted by the chemistry laboratory. On the other hand, the RIA and the Beckman Coulter Access methods for measuring \text{uE}_3 for this set of data did not demonstrate good agreement and the Beckman Coulter Access \text{uE}_3 was not adopted.

ACKNOWLEDGEMENT

We wish to acknowledge Randy Robinson MT(ASCP), Susie Rogers MT(ASCP), and Carole Mahaffey MT(ASCP), WVU Hospitals Inc, for the laboratory data and their assistance.

REFERENCES


### Table 1. Comparison of Photon ERA PSA to Access 1 PSA

<table>
<thead>
<tr>
<th>Concentration range, µg/L</th>
<th>n</th>
<th>Mean, ng/mL:</th>
<th>r</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ERA</td>
<td>Access</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>35</td>
<td>1.1</td>
<td>1.1</td>
<td>0.9905</td>
<td>0.0800</td>
<td>0.9584</td>
</tr>
<tr>
<td>0-10</td>
<td>37</td>
<td>1.5</td>
<td>1.5</td>
<td>0.9776</td>
<td>0.0393</td>
<td>1.0008</td>
</tr>
<tr>
<td>0-105</td>
<td>40</td>
<td>7.0</td>
<td>6.7</td>
<td>0.9996</td>
<td>0.1629</td>
<td>0.9374</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of Photon ERA PSA to Access 2 PSA

<table>
<thead>
<tr>
<th>Concentration range, µg/L</th>
<th>n</th>
<th>Mean, µg/L:</th>
<th>r</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ERA</td>
<td>Access</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>35</td>
<td>1.1</td>
<td>1.1</td>
<td>0.9847</td>
<td>0.0811</td>
<td>0.9678</td>
</tr>
<tr>
<td>0-10</td>
<td>37</td>
<td>1.0</td>
<td>1.5</td>
<td>0.9964</td>
<td>0.0486</td>
<td>1.0019</td>
</tr>
<tr>
<td>0-105</td>
<td>40</td>
<td>7.0</td>
<td>7.0</td>
<td>0.9998</td>
<td>0.1120</td>
<td>0.9958</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of RIA uE₃ to Access 1 uE₃

<table>
<thead>
<tr>
<th>Concentration range, µmol/L</th>
<th>n</th>
<th>Mean, µmol/L:</th>
<th>r</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>Access</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7</td>
<td>33</td>
<td>3.14</td>
<td>4.05</td>
<td>0.8696</td>
<td>-0.3741</td>
<td>1.4105</td>
</tr>
</tbody>
</table>

### Table 4. Comparison of RIA uE₃ to Access 2 uE₃

<table>
<thead>
<tr>
<th>Concentration range, µmol/L</th>
<th>n</th>
<th>Mean, µmol/L:</th>
<th>r</th>
<th>Intercept</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>Access</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-7</td>
<td>33</td>
<td>3.14</td>
<td>3.90</td>
<td>0.8643</td>
<td>-0.2292</td>
<td>1.3150</td>
</tr>
</tbody>
</table>

Validity of Injecting Drug Users’ Self Report of Hepatitis A, B, and C

ERIN G SCHLICTING, MARK E JOHNSON, CHRISTIANE BREMS, REBECCA S WELLS, DENNIS G FISHER, GRACE REYNOLDS

OBJECTIVE: To test the validity of drug users self-reports of diseases associated with drug use, in this case hepatitis A, B, and C.

DESIGN: Injecting drug users (n = 653) were recruited and asked whether they had been diagnosed previously with hepatitis A, B, and/or C. These self-report data were compared to total hepatitis A antibody, hepatitis B core antibody, and hepatitis C antibody seromarkers as a means of determining the validity of the self-reported information.

SETTING: Anchorage, Alaska.

PARTICIPANTS: Criteria for inclusion included being at least 18-years old; testing positive on urinalysis for cocaine metabolites, amphetamine, or morphine; having visible signs of injection (track marks).

INTERVENTION: Serological testing for hepatitis A, B, and C.

MAIN OUTCOME: Findings indicate high specificity, low sensitivity, and low kappa coefficients for all three self-report measures.

RESULTS: Subgroup analyses revealed significant differences in sensitivity associated with previous substance abuse treatment experience for hepatitis B self-report and with gender for hepatitis C self-report.

CONCLUSION: Given the low sensitivity, the validity of drug users’ self-reported information on hepatitis should be considered with caution.

ABBREVIATIONS: HAV = hepatitis A virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IDU = injection drug user; STD = sexually transmitted disease.

INDEX TERMS: hepatitis; injection drug use; infectious diseases; self-report; validity.

Clin Lab Sci 2003;16(2):99

Erin G Schlicting is a doctoral student, University of Rhode Island, Kingston RI.

Mark E Johnson is Professor of Psychology at the University of Alaska, Anchorage AK.

Christiane Brems is Professor of Psychology at the University of Alaska, Anchorage AK.

Rebecca S Wells is at the Alaska Native Health Board, Anchorage AK.

Dennis G Fisher is Director, Center for Behavioral Research and Services at California State University, Long Beach CA.

Grace Reynolds is Associate Director, Center for Behavioral Research and Services at California State University, Long Beach CA.

Address for correspondence: Dennis G Fisher PhD, Director, Center for Behavioral Research and Services, 1090 Atlantic Avenue, Long Beach CA 90813. (562) 495-2330 x121. (562) 983-1421 (fax). dfisher@csulb.edu

Many major national substance abuse treatment outcome studies, sexually transmitted disease (STD) prevention programs, publicly-funded drug treatment and prevention projects, and other substance abuse-related programs rely heavily, if not exclusively, on information gathered via drug users’ self-reports. Often major policy or intervention decisions are made based on the data obtained from such studies or efforts. Given that self-report is a commonly used tool for collecting information on drug use, sexual behaviors, STDs,
Individuals who inject drugs may over- or under-report information for a variety of reasons. For example, given that the information being collected, e.g., drug use, sexual behaviors, and STD information, is often perceived as socially stigmatizing, injection drug users (IDUs) may feel uncomfortable reporting the behavior, leading to under-reporting. On the other hand, drug users may over-report behaviors if they feel excessive reporting will be advantageous for them, e.g., by helping them gain enrollment to an incentive-based research project or priority for admission to a substance abuse treatment program. Many other variables, such as intrinsic motivation to complete a research or diagnostic interview, failure to recall past events, fear of legal reprisal, insufficient description of reported events, and cognitive impairment may bias the validity of self-reported information collected from IDUs.

The accuracy and validity of drug users’ self-report is of special concern when the information being collected is disease or health-related. The lifestyles of IDUs place them at great risk for contracting sexually transmitted diseases and other infectious diseases that are transmitted through close interpersonal contact and poor living conditions. Diseases of primary concern are human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), hepatitis A (HAV), B (HBV) and C (HCV), and STDs, e.g., chlamydia, gonorrhea, herpes, and syphilis, all of which have a high prevalence among injecting drug users and have the potential to spread to the general population. The risk to both IDUs and the general population highlights the importance of having accurate information about the disease histories of IDUs.

Studies that have compared self-report information from IDUs regarding existing infectious diseases such as oral and genital herpes or syphilis with laboratory analysis found low correlations between these two sources of information. Such findings contrast other studies that reported high levels of validity of self-report when inquiring about more general information about high-risk behaviors, e.g., injecting practices or sexual behaviors. One possible explanation for this discrepancy may be that infected individuals may have experienced symptoms or received a diagnosis months or years before data about infection were collected, introducing the possibility of recall error. Alternatively, validity of self-report information on STDs may be influenced by the social stigma surrounding such infection.

In the United States, hepatitis A is one of the more commonly reported, vaccine-preventable diseases. IDUs have a higher prevalence of HAV than the general population. HAV may be transmitted by injection but fecal contamination of the illicit drugs or the poor hygienic conditions common to IDUs are more likely to be the transmission route. Hepatitis B is the most common cause of acute and chronic liver disease and a significant public health problem in the U.S. and all regions of the world. HBV is transmitted through sexual encounters, blood-to-blood contact, and from an infected mother to her infant. Hepatitis C has recently emerged as a major public health concern. HCV is transmitted through blood-to-blood contact and from infected mother to her infant. Because injection drug users often engage in high-risk behaviors that facilitate transmission of infectious disease such as needle sharing and unsafe sex practices, they are at high risk for contracting HAV, HBV, and HCV. Indeed, IDUs account for most new HCV cases reported in the U.S. The high risk of hepatitis transmission among IDUs highlights the need for valid information about HAV, HBV, and HCV infection status. Accurate assessment of hepatitis incidence in this population can assist public health professionals and researchers create better plans to decrease the risk and incidence of infection.

Prior research has found limited validity for self-report HBV data and has not explored HAV and HCV self-report. Fisher, Kuhrt-Hunstiger, Orr, and Davis found low levels of validity for self-report of hepatitis B infection. However, high levels of validity for self-report of no infection were found, indicating valid self-reporting of individuals who are not infected with HBV. In the current study, the accuracy of IDU’s self-reported information on HAV, HBV, and HCV infection was compared with actual serostatus as obtained through serological blood testing. Further, participant gender, ethnicity, and history of substance abuse treatment were considered as possible moderating variables of self-report validity. Primary methods used to determine validity of the self-report data were sensitivity and specificity. Sensitivity is defined as “the ability of a test to identify correctly those who have the disease”; specificity refers to “the ability of a test to identify correctly those who do not have the disease.”
METHOD

Participants
The total sample consisted of 497 male and 156 female injection drug users participating in a National Institute on Drug Abuse (NIDA) project designed to evaluate the effectiveness of a needle exchange program in reducing the incidence of blood-borne infections. Criteria for inclusion in the study were being of age 18 years or older; possession of picture identification; testing positive on urinalysis for cocaine metabolites, amphetamine, or morphine; having visible signs of injection; and self-report of recent injection. Of the participants, 363 (56%) were Caucasian, 135 (21%) Native American/Alaska Native, 122 (19%) African American, 23 (4%) Hispanic, 6 (0.9%) Asian American, and 4 (0.6%) Other. Ages of the participants ranged from 18 to 66 years with a mean age of 38.0 years (SD = 7.9). Relative to prior substance abuse treatment, 245 (37%) reported no prior treatment, 82 (13%) reported outpatient treatment (including in prison and methadone maintenance), and 326 (50%) reported both outpatient and inpatient treatment. There were no reports of inpatient treatment without outpatient treatment.

Risk behavior assessment (RBA)23
As part of their involvement in this research project, participants were administered the Risk Behavior Assessment, a structured interview developed by the Community Research Branch of NIDA in collaboration with the Cooperative Agreement for AIDS Community-Based Outreach/Intervention Research Program grantees. Trained interviewers read items to participants that requested information about demographics; HIV risk behaviors, such as drug use, needle sharing, and sexual behaviors; drug treatment history; health history and status; and work and income. The RBA has been demonstrated to have very good reliability and validity for HIV sexual and drug use questions.2,9,24,25 In addition, reliability for items pertaining to work and income was found to be good.26 For the current study, the RBA question of interest was, “How many times have you been told by a doctor or nurse that you had hepatitis B?” The 48-hour test-retest reliability for this question is 0.91.21,24 Self-report of HAV and HCV was obtained using a supplemental questionnaire that asked “How many times have you been told by a doctor, nurse, or health counselor that you have hepatitis A?” and “How many times have you been told by a doctor, nurse, or health counselor that you had hepatitis C?”

Hepatitis serostatus
As part of their regular participation in the needle exchange project, participants received pretest serological counseling for hepatitis A, B, and C, and HIV. Blood was then drawn by a certified phlebotomist. Blood samples were tested for HAV, HBV, and HCV seromarkers. The test for hepatitis A was the HAVAB® EIA enzyme immunoassay of total antibody; for hepatitis B core antigen was Corzyme® enzyme immunoassay; and for hepatitis C, the Abbott HCV EIA 2.0 enzyme immunoassay for antibody was used.27,28,29 Participants were considered to be infected with HBV if the test results were core (Anti-HBc) positive. Core antibody is a lifelong marker that indicates past exposure to HBV.

PROCEDURE
Participants were recruited using targeted sampling and snowball sampling that integrated various efforts including word of mouth, flyers posted on bulletin boards at homeless shelters, and the use of outreach workers.30,31 Participants were informed of eligibility requirements and the purpose of the study before enrollment was granted; informed consent was obtained prior to the first interview. Urine samples were acquired to determine eligibility for participation in the study. After obtaining informed consent, the RBA was administered and pretest counseling was provided. Blood samples were then obtained and sera tested for HIV and hepatitis. Following completion of the interview, participants were paid for their participation. Later, participants returned to obtain their serological test results and received posttest counseling. Of the 653 participants, 477 were tested for hepatitis A, 550 for hepatitis B, and 558 for hepatitis C.

Statistical analysis
Sensitivity and specificity
The serostatus of the individual was used as the true indicator for disease, while the self-report from the RBA or supplemental hepatitis questionnaire were referred to as the clinical tests. Sensitivity of the self-reported information was calculated as the proportion of participants who tested positive for a given seromarker who also self-reported having been told that they had that disease. Specificity of the self-reported information was calculated as the proportion of participants who tested negative for a given seromarker who self-reported that they had never been told they had that disease.

Subgroup analysis
Previous research indicates variables such as gender, ethnicity, and previous treatment involvement may influence the va-
lidity of information self-reported by injection drug users.\textsuperscript{21,32} Data from the participants in the current study were examined on the subgroup level to determine whether the variables of gender, ethnicity, and previous treatment experience influenced the validity of the responses. Subgroup analysis was performed using a series of binomial tests of proportions, comparing within gender, ethnicity, and previous treatment experience. Analyses based on ethnicity included only the three groups with adequate sample sizes Native American/Alaska Native, Caucasian, and African American.

**Kappa**
The reliability or agreement between self-reported infection and serological results was analyzed using Cohen’s kappa.\textsuperscript{33} Kappa measures agreement between two variables beyond that expected to occur by chance alone, and is commonly used in validity studies.\textsuperscript{2} A maximum value of 1.00 indicates perfect agreement. As obtained Cohen’s kappa distributions can be affected by imbalances in marginal totals as were found in the current analyses, two other indices are provided to help clarify the actual reliability of the items being analyzed.\textsuperscript{34,35} These two indices are $p_{pos}$, observed proportion of positive agreement and $p_{neg}$, observed proportion of negative agreement.

**RESULTS**

**Sensitivity and specificity**
Table 1 provides the results of self-report and serological testing. Of the 477 participants with a baseline HAV blood test, 31 reported having previously been told that they were infected with HAV, for a prevalence of 6.5%. Serological testing revealed 155 participants as HAV positive, for a true prevalence of 32.5%. Of the 155 participants who tested positive for HAV, 21 reported that they had been told they were infected with HAV (sensitivity = 13.5%). Of the 322 participants who tested negative for HAV, 312 reported a doctor or nurse had never told them they were infected with HAV.

<table>
<thead>
<tr>
<th>Table 1. Hepatitis self-report and serological results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAV</strong></td>
</tr>
<tr>
<td>Self-report</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>HBV</strong></td>
</tr>
<tr>
<td>Self-report</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>HCV</strong></td>
</tr>
<tr>
<td>Self-report</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Sensitivity and specificity for HAV by subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>95%</strong></td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
</tr>
<tr>
<td>African American</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td>Native American/Alaska Native</td>
</tr>
<tr>
<td><strong>Prior treatment</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Outpatient</td>
</tr>
<tr>
<td>Inpatient</td>
</tr>
</tbody>
</table>
HAV (specificity = 96.89%). Of the 550 participants with a baseline HBV blood test, 82 reported having previously been told that they were infected with HBV, for a prevalence of 14.9%. Serological testing revealed 219 participants to be HBV positive, for a true prevalence of 39.8%. Of the 219 participants who tested positive for HBV, 67 reported that they had been told they were infected with HBV (sensitivity = 30.59%). Of the 331 participants who tested negative for HBV, 316 reported a doctor or nurse had never told them they were infected with HBV (specificity = 95.47%). Of the 557 participants with a baseline HCV test, 74 reported having been told that they were infected with HCV, for a prevalence of 13.3%. Serological testing revealed 293 individuals to be HCV positive for a true prevalence of 52.70%. The sensitivity of the HCV self report was 23.5%, with 69 of the 293 infected individuals self-reporting their HCV positive status. Specificity was 98.0%, with 258 of 263 correctly reporting their HCV negative status.

Subgroup analysis
Tables 2, 3, and 4 provide the results of the analyses examining possible differences in sensitivity and specificity when broken down separately by participant gender, ethnicity, and prior treatment experience. For self-report of HAV, no significant differences were revealed in sensitivity or specificity relative to gender, ethnicity, or treatment experience. For self-report of HBV, significant differences in sensitivity were associated only with previous substance abuse treatment experience, with a greater proportion of participants infected with HBV with previous inpatient substance abuse treatment experience (46/118 = 38.98%) reporting HBV infection than participants without previous drug treatment (11/70 = 15.71%), p < .05. No significant differences in specificity were revealed between outpatient and inpatient treatment. No significant differences were revealed in sensitivity or specificity relative to participant gender or ethnicity. For HCV, significant differences in sensitivity were revealed only between women (28/75; 37.33%) and men (41/218; 18.81%), with women having greater sensitivity scores, p < .001. No differences in specificity of HCV self-report were found with respect to gender, and there were no differences in sensitivity or specificity with respect to ethnicity or treatment experience.

Kappa
As revealed in Table 5, kappa statistics were all consistently very low, with overall statistics ranging from .13 to .28, indicating low reliability. The ppos and pneg provide more detailed statistics regarding kappa statistics and reveal the same pattern as identified by sensitivity and specificity, that is, low probability of accurately reporting positive results and high probability of accurately reporting negative results.

DISCUSSION
As indicated by the low sensitivity, results from the current study revealed a significant discrepancy between IDUs’ self-reported HAV, HBC, and HCV infection status and the results of serological testing for markers of infection with the corresponding hepatitis virus. Specifically, the results revealed self-reported prevalence to be 6.5% for HAV, 14.9% for

---

**Table 3. Sensitivity and specificity for HBV by subgroup**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>95% Confidence Interval</th>
<th>Specificity</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>30.59</td>
<td>24.56 – 37.16</td>
<td>95.47</td>
<td>92.64 – 97.44</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26.75</td>
<td>20.01 – 34.39</td>
<td>95.91</td>
<td>92.80 – 97.94</td>
</tr>
<tr>
<td>Female</td>
<td>40.32</td>
<td>28.05 – 53.55</td>
<td>93.55</td>
<td>84.30 – 98.21</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>25.00</td>
<td>12.12 – 42.20</td>
<td>93.55</td>
<td>84.30 – 98.21</td>
</tr>
<tr>
<td>Caucasian</td>
<td>32.81</td>
<td>24.78 – 41.67</td>
<td>96.15</td>
<td>92.24 – 98.44</td>
</tr>
<tr>
<td>Native American/Alaska Native</td>
<td>26.19</td>
<td>13.86 – 42.04</td>
<td>94.29</td>
<td>86.01 – 98.42</td>
</tr>
<tr>
<td><strong>Prior treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15.71</td>
<td>8.11 – 26.38</td>
<td>98.68</td>
<td>90.84 – 98.40</td>
</tr>
<tr>
<td>Outpatient</td>
<td>32.26</td>
<td>16.68 – 51.37</td>
<td>91.89</td>
<td>78.09 – 98.30</td>
</tr>
<tr>
<td>Inpatient</td>
<td>38.98</td>
<td>30.14 – 48.39</td>
<td>96.13</td>
<td>91.77 – 98.57</td>
</tr>
</tbody>
</table>
HBV, 13.3% for HCV, as compared to true prevalence of 32.5%, 39.8%, and 52.7%, respectively. This significant discrepancy, or low sensitivity of self-report, indicates that IDUs’ self-reports of hepatitis infection are biased underestimates and should only be used for estimating a lower bound of the true prevalence of infection.

Although sensitivity of self-report regarding infection with a hepatitis virus is low, specificity of self-report was high. Those participants who reported having never been told by a doctor or nurse that they were infected with a given hepatitis virus were very accurate in reporting this information, as indicated by specificity rates that ranged from 95.5% for HBV to 97% for HAV to 98.1% for HCV. The high specificity findings support other findings regarding self-report by drug users that suggest that self-report tends to be valid when the variable of interest is one of which the drug user has direct knowledge.2,24,36-39 For example, individuals who have used illicit substances in the previous 48 hours are likely to provide reasonably valid self-report of recent drug use because of their direct and recent knowledge of the use.

Two major issues may explain the low sensitivity of self-reported hepatitis infection. First and foremost, infected individuals may not have been aware of their actual serostatus. Such lack of awareness is likely given that the IDUs in the current study may have poor access to healthcare due to their low socioeconomic status, i.e., 46% reported having earned less than $500 in the last 30 days. Due to inaccessible healthcare, participants may have been less inclined to seek medical assistance when the initial symptoms of hepatitis infection appeared and may never have been diagnosed. Further, symptoms of hepatitis infection are often flu-like, including nausea and fatigue, symptoms that may be interpreted by an IDU as withdrawal symptoms. When experiencing these symptoms, the individual may choose to wait for the symptoms to pass or to self-medicate through the use of illicit substances. Finally, individuals using illicit substances may refrain from seeking medical care altogether simply for fear of possible legal consequences for their drug use.

A second possible explanation for the low sensitivity of self-report may be that participants may have had external or internal motivations to under report infection. A possible external influence may have been the perception of denial as socially desirable and admission as socially stigmatizing. Internal factors may have included a motivation to get through the interview more quickly, difficulty recalling past events, or cognitive impairment. Given the nature of the information being reported by the participant, social desirability is a likely external factor. For example, hepatitis B is considered by many to be a stigmatized sexually transmitted disease. To avoid this stigma, participants may underreport HBV. Regarding internal factors, recall bias can have significant effects on self-reported information, particularly if the event of interest occurred more than 30 days prior to the interview. Further complicating recall among IDUs is the possibility that the participant may have been under the influence of illicit substances either at the time of notification of hepatitis infection or during the interview conducted for the current study.

| Table 4. Sensitivity and specificity for HCV, by subgroup |
|---------------------------------|------------|-----------------|-----------------|-----------------|
|                                 | Sensitivity | 95% Confidence Interval | Specificity | 95% Confidence Interval |
| Total                           | 23.55       | 18.81 – 28.83     | 98.10       | 95.62 – 99.38      |
| Gender                          |             |                  |              |                  |
| Male                            | 18.81       | 13.85 – 24.64     | 98.12       | 95.26 – 99.49     |
| Female                          | 37.33       | 26.43 – 49.27     | 98.00       | 89.35 – 99.95     |
| Ethnicity                       |             |                  |              |                  |
| African American                | 23.81       | 12.05 – 39.45     | 100.00      | 94.13 – ——       |
| Caucasian                       | 25.52       | 19.52 – 32.30     | 95.80       | 90.47 – 98.62     |
| Native American/Alaska Native   | 20.93       | 10.04 – 36.04     | 100.00      | 94.79 – ——       |
| Prior treatment                 |             |                  |              |                  |
| None                            | 17.31       | 10.59 – 25.97     | 98.25       | 93.81 – 99.79     |
| Outpatient                      | 25.00       | 13.19 – 40.34     | 100.00      | 84.56 – ——       |
| Inpatient                       | 27.59       | 20.50 – 35.62     | 97.64       | 93.25 – 99.51     |
Individuals who were previously enrolled in either outpatient or inpatient substance abuse treatment and were HBV seropositive had higher sensitivity rates. This finding may be accounted for by the fact that individuals in drug treatment are commonly required to have a physical examination during the process of enrollment into the program. In such a physical examination, the healthcare professional will typically include questions about symptoms that might indicate possible HBV infection. Further, given the high prevalence of blood-borne pathogens such as HBV and HIV among drug users, screens for these pathogens are often included in such a physical examination. In the absence of symptoms, these blood screens may detect HBV seromarkers or elevated liver enzymes that could indicate possible HBV infection. For all of these reasons and others, those participants with previous substance treatment experience may be more likely to have been told they were infected with HBV, resulting in more accurate self-reporting of HBV infection.

The fact that it is not clear whether sensitivity of self-report is low because participants denied their infection status or were unaware of it is the most significant limitation to be considered when interpreting the results of this project. Future research will need to address this issue. One possible method for doing so would be to recruit participants from a previous research project in which serostatus testing and feedback was conducted and report about each participant. Another means would be to recruit participants from clinics at which they had been notified of their hepatitis serostatus.

Although it appears that the IDUs in the current study reported accurately when they were aware of infection, it would appear that awareness or knowledge of infection is limited, at least in this population. These findings highlight the need for increased efforts at providing hepatitis testing as part of the enrollment process for substance abuse treatment and outreach services to out-of-treatment drug users. The need for such increased efforts is strengthened by the very high rates of hepatitis infection revealed in this study, rates that are significantly higher than would be suggested if relying strictly upon self-report data.

This study was supported by the following grants from the National Institute on Drug Abuse: U01DA07290 and R01DA10181.

REFERENCES


Table 5. Cohen’s kappa, p_pos, and p_neg, broken down by subgroup

<table>
<thead>
<tr>
<th></th>
<th>HAV</th>
<th></th>
<th></th>
<th>HAV</th>
<th></th>
<th></th>
<th>HAV</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kappa</td>
<td>p_pos</td>
<td>p_neg</td>
<td>kappa</td>
<td>p_pos</td>
<td>p_neg</td>
<td>kappa</td>
<td>p_pos</td>
<td>p_neg</td>
</tr>
<tr>
<td>Total</td>
<td>.13</td>
<td>.23</td>
<td>.81</td>
<td>.29</td>
<td>.45</td>
<td>.79</td>
<td>.21</td>
<td>.38</td>
<td>.69</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>.13</td>
<td>.21</td>
<td>.82</td>
<td>.26</td>
<td>.40</td>
<td>.80</td>
<td>.17</td>
<td>.31</td>
<td>.70</td>
</tr>
<tr>
<td>Female</td>
<td>.14</td>
<td>.28</td>
<td>.77</td>
<td>.34</td>
<td>.55</td>
<td>.74</td>
<td>.31</td>
<td>.54</td>
<td>.67</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>.03</td>
<td>.05</td>
<td>.75</td>
<td>.21</td>
<td>.37</td>
<td>.79</td>
<td>.27</td>
<td>.38</td>
<td>.79</td>
</tr>
<tr>
<td>Caucasian</td>
<td>.23</td>
<td>.33</td>
<td>.85</td>
<td>.32</td>
<td>.47</td>
<td>.79</td>
<td>.18</td>
<td>.40</td>
<td>.61</td>
</tr>
<tr>
<td>Native American/Alaska Native</td>
<td>.08</td>
<td>.17</td>
<td>.80</td>
<td>.24</td>
<td>.39</td>
<td>.79</td>
<td>.25</td>
<td>.35</td>
<td>.80</td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>.12</td>
<td>.17</td>
<td>.82</td>
<td>.14</td>
<td>.25</td>
<td>.80</td>
<td>.16</td>
<td>.29</td>
<td>.72</td>
</tr>
<tr>
<td>Outpatient</td>
<td>.15</td>
<td>.25</td>
<td>.80</td>
<td>.25</td>
<td>.45</td>
<td>.74</td>
<td>.18</td>
<td>.40</td>
<td>.57</td>
</tr>
<tr>
<td>Inpatient</td>
<td>.14</td>
<td>.26</td>
<td>.81</td>
<td>.38</td>
<td>.54</td>
<td>.79</td>
<td>.24</td>
<td>.43</td>
<td>.70</td>
</tr>
</tbody>
</table>


27. Abbott Laboratories Diagnostics Division. Enzyme immunoassay for the qualitative detection or semi-quantification of total antibody to hepatitis A virus (anti-HAV) in human serum or plasma; 1991.

28. Abbott Laboratories Diagnostics Division. Enzyme immunoassay for the qualitative determination of total antibody to hepatitis B virus core antigen in serum or plasma; 1995.


Managing the Bleeding Patient

GEORGE A FRITSMA

ABBREVIATIONS: PT = prothrombin time; PTT = partial thromboplastin time.

INDEX TERMS: Anatomic hemorrhage; coagulopathy; systemic hemorrhage.

Clin Lab Sci 2003;16(2):107

George A Fritsma MS MT(ASCP) is in the Department of Pathology, UAB Coagulation Service Coordinator at the University of Alabama at Birmingham, Birmingham AL.

Address for correspondence: George A Fritsma MS MT (ASCP), Department of Pathology, 619 South 19th Street, West Pavilion, P230, University of Alabama at Birmingham, Birmingham AL 35249, gfritsma@path.uab.edu. Website: http://uabcoag.net

George A Fritsma is the Focus: Hemorrhagic Abnormalities guest editor.

Focus Continuing Education Credit: see pages 123 to 126 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES (for the entire section)
The reader will be able to:
1. distinguish between anatomic and systemic hemorrhage.
2. distinguish between acquired and congenital hemorrhage.
3. select hemostatic laboratory tests that may be used to establish the presence of a hemostatic disorder.
4. identify hemostatic laboratory tests for use in pinpointing the cause of hemorrhage.
5. interpret the laboratory test profile results used to establish the presence of disseminated intravascular coagulation.
6. interpret the laboratory test profile results used to establish the presence of von Willebrand disease.
7. select single coagulation factor assays.
8. describe how to perform and interpret mixing studies.
9. determine the presence of a coagulation inhibitor.
10. select the appropriate coagulation factor concentrate therapy to treat hemorrhagic disorders.
11. calculate the correct dosage of coagulation factor concentrate to appropriately treat hemorrhagic disorders.
12. assess the dosage adequacy of the coagulation factor concentrate used to treat hemorrhagic disorders.
13. detail the selection and dosage of factor concentrates for von Willebrand disease, hemophilia, and hemophilia with the presence of an inhibitor.

The acute care hemostasis laboratory must be equipped to manage both acute and chronic hemorrhage. Hemorrhage is defined as bleeding that can be arrested only by special interventions such as pressure, elevation, ice, cauterization, ligation, or therapy. Therapy may include non-biologic drugs such as DDAVP and Amicar, and coagulation concentrates in various forms. Hemorrhage may be local or general, anatomic or systemic, acquired or congenital. To establish the cause for a hemorrhagic event, the clinician first completes a history and physical examination, and then follows up with diagnostic laboratory tests.

LOCAL VERSUS GENERAL HEMORRHAGE
Most bleeding is local. Hemorrhage from a single location signals a trauma, tissue necrosis, or a blood vessel defect. A surgical site may bleed because of an inadequately ligated or cauterized vessel. Local bleeding seldom implies a coagulopathy.

Congenital coagulopathies are uncommon, occurring in approximately one in 800 individuals, and are usually detected in infancy. Patients often have relatives with similar
hemorrhagic symptoms. Hemorrhages may be spontaneous and may occur in unexpected locations such as joints, body cavities, retinal veins and arteries, or the central nervous system. Patients with mild congenital hemorrhagic disorders may have no symptoms until they reach adulthood or when they experience some physical challenge such as sports activity, a trauma, dental extraction, or surgery. The most common congenital deficiencies are von Willebrand disease, platelet function disorders, and factor VIII, IX, or XI deficiencies. Inherited fibrinogen, prothrombin, or factor V, VII, X, or XIII deficiencies are rare (Table 3).

ANATOMIC VERSUS SYSTEMIC HEMORRHAGE

General hemorrhage may be anatomic or systemic. Anatomic hemorrhage is seen in acquired or congenital plasma procoagulant deficiencies. When there is anatomic hemorrhage, bleeds may immediately follow traumatic events, but are often delayed or recurrent. Some bleeding is spontaneous. Most anatomic bleeds are internal: bleeding into joints, body cavities, or the central nervous system. Joint bleeds cause swelling, acute pain, and inflammation. Bleeds into soft tissues, such as muscles or fat, cause nerve compression and subsequent loss of function, temporary or permanent.6 Bleeds into body cavities cause symptoms related to the or-

FOCUS: HEMORRHAGIC ABNORMALITIES

<table>
<thead>
<tr>
<th>Table 1. General hemorrhage symptoms that suggest a coagulopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptom</strong></td>
</tr>
<tr>
<td>Excessive bleeding</td>
</tr>
<tr>
<td>Menorrhagia</td>
</tr>
<tr>
<td>Epistaxis</td>
</tr>
<tr>
<td>Hematemesis</td>
</tr>
<tr>
<td>Bleeding from gums and mucus membranes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Disorders that cause a secondary coagulopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disorder</strong></td>
</tr>
<tr>
<td>End stage liver disease with hepatosplenomegaly</td>
</tr>
<tr>
<td>Uremia</td>
</tr>
<tr>
<td>Malnutrition</td>
</tr>
<tr>
<td>Immune thrombocytopenia</td>
</tr>
<tr>
<td>Thrombotic thrombocytopenic purpura</td>
</tr>
<tr>
<td>Aplastic anemia, acute leukemia and myelodysplastic syndromes</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
</tr>
</tbody>
</table>
gan that is affected, for instance, bleeding into the central nervous system causes headache, confusion, seizures, and coma; these must be managed as medical emergencies. Bleeds in the kidney cause hematuria and kidney failure.

Systemic, or mucosal, hemorrhage includes petechiae, purpura, easy bruising, epistaxis, menorrhagia, hematuria, hematemesis, and gingival bleeding. Systemic hemorrhage associates with thrombocytopenia, qualitative platelet disorders, mild or moderate von Willebrand disease, or vascular disorders such as telengectasia. A careful history and physical examination may distinguish between anatomic and systemic bleeding; the distinction helps direct investigative testing and treatment.

Liver disease, severe von Willebrand disease, and DIC are accompanied by both anatomic and systemic bleeding. Uremia usually causes systemic bleeding and malnutrition, anatomic bleeding.

LABORATORY TESTS IN GENERAL HEMORRHAGE

Please refer to the accompanying article in this issue by Laura J Taylor, “Laboratory Management of Hemorrhage” for a discussion of hemostasis laboratory testing for the bleeding patient.

When the history and physical examination lead the physician to suspect a hemostatic disorder, the three primary assays used are prothrombin time (PT), partial thromboplastin time (PTT), and platelet count (Table 4). When there is anatomic hemorrhage and either the PT or PTT result is prolonged to 1.5 times the mean of the reference interval, a procoagulant deficiency or specific inhibitor is suspected and follow-up work begins. Mixing studies and factor assays help establish the cause for bleeding, most often an acquired multiple factor deficiency or an antibody such as anti-VIII. The thrombin clotting time is used in to rule out plasma heparin, often unreported.

Systemic bleeding accompanied by a platelet count less than 50 x 10^9/L prompts follow-up testing for thrombocytopenia, whereas if the count is 150 x 10^9/L or higher, von Willebrand disease or a qualitative platelet abnormality is suspected.

When bleeding is both systemic and anatomic, and the platelet count is below the established reference interval, disseminated intravascular coagulation may be confirmed by fibrinogen and D-dimer assays.

TREATMENT OF GENERAL HEMORRHAGE

Please refer to the accompanying article in this issue by Margaret G Fritsma, “Use of Blood Products and Factor Concentrates for Coagulation Therapy” for a discussion of the use of plasma and factor concentrates.

If the cause for the general hemorrhage is multiple factor deficiency as may be seen in liver disease, the treatment is fresh frozen plasma or, in limited cases, cryoprecipitate (Table 5). Platelet concentrate is used when the platelet count drops to life-threatening levels, typically below 20 x 10^9/L, though some physicians use 10 x 10^9/L.

<table>
<thead>
<tr>
<th>Table 3. Congenital bleeding disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder</td>
</tr>
<tr>
<td>Von Willebrand disease</td>
</tr>
<tr>
<td>Congenital thrombocytopenia</td>
</tr>
<tr>
<td>Congenital platelet function disorders</td>
</tr>
<tr>
<td>Factor VIII deficiency</td>
</tr>
<tr>
<td>Factor IX deficiency</td>
</tr>
<tr>
<td>Factor XI deficiency</td>
</tr>
<tr>
<td>Fibrinogen, factor II, V, VII, X, or XIII</td>
</tr>
</tbody>
</table>
Severe von Willebrand disease requires fractionated factor VIII concentrates that contain von Willebrand factor, whereas the acute bleeding associated with hemophilia requires single factor concentrates prepared either by monoclonal plasma purification or recombinant manufacturing techniques. When an inhibitor to a coagulation factor such as anti-factor VIII, is detected, prothrombin complex concentrates, activated prothrombin complex concentrates, or recombinant factor VIIa are effective.

**COMMUNICATION BETWEEN THE CLINICIAN AND THE LABORATORY**

Clinical decision-making is based upon established practice models. An academic approach to clinical practice provides a useful framework. Nevertheless, clinical situations arise in which individualized judgment is essential. Refer to the accompanying article in this issue by Marisa B Marques MD titled “Treatment of Single Factor Deficiencies: A Case-Study Approach” for a practical look at the clinical management of hemorrhage. The successful management of the bleeding patient ultimately depends upon full communication among the clinician, the hemostasis laboratory, and the transfusion service.

**REFERENCES**


<table>
<thead>
<tr>
<th>Table 4. Laboratory tests in hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Tests</strong></td>
</tr>
<tr>
<td>PT, PTT, Platelet count</td>
</tr>
<tr>
<td><strong>Multiple coagulation factor deficiency</strong></td>
</tr>
<tr>
<td>Mixing studies, factor assays, thrombin time, platelet count</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
</tr>
<tr>
<td>Bone marrow examination, platelet antibodies</td>
</tr>
<tr>
<td><strong>Qualitative platelet disorder</strong></td>
</tr>
<tr>
<td>Platelet aggregometry, PFA-100</td>
</tr>
<tr>
<td><strong>Von Willebrand disease</strong></td>
</tr>
<tr>
<td>VWF activity, VWF antigen, and factor VIII</td>
</tr>
<tr>
<td><strong>Disseminated intravascular coagulation</strong></td>
</tr>
<tr>
<td>Fibrinogen, D-dimer</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5: Treatment of general hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multiple factor deficiency secondary to liver disease, renal disease, or malnutrition</strong></td>
</tr>
<tr>
<td>Fresh frozen plasma, cryoprecipitate</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
</tr>
<tr>
<td>Platelet concentrate</td>
</tr>
<tr>
<td><strong>Von Willebrand disease</strong></td>
</tr>
<tr>
<td>Fractionated plasma factor VIII preparaations</td>
</tr>
<tr>
<td><strong>Single factor deficiency (hemophilia)</strong></td>
</tr>
<tr>
<td>Monoclonally purified plasma-derived factor concentrate; recombinant factor concentrate</td>
</tr>
<tr>
<td><strong>Factor inhibitor</strong></td>
</tr>
<tr>
<td>Prothrombin complex, activated prothrombin complex, recombinant activated factor VII</td>
</tr>
</tbody>
</table>

110 VOL 16, NO 2  SPRING 2003  CLINICAL LABORATORY SCIENCE
ABBREVIATIONS: APTT = activated partial thromboplastin time; DIC = disseminated intravascular coagulation; PT = prothrombin time; PTT = partial thromboplastin time; TCT = thrombin clotting time; vWD = von Willebrand disease; vWF = von Willebrand factor.

INDEX TERMS: Disseminated intravascular coagulation; partial thromboplastin time; platelet count; prothrombin time; von Willebrand disease.

Laura J Taylor MT(ASCP) is a medical technologist at the University of Alabama at Birmingham, Birmingham AL.

Address for correspondence: Laura J Taylor MT(ASCP), Department of Pathology, 619 South 19th Street, West Pavilion, P230, University of Alabama at Birmingham, Birmingham AL 35233. http://uabcoag.net

George A Fritsma is the Focus: Hemorrhagic Abnormalities guest editor.

Focus Continuing Education Credit: see pages 123 to 126 for learning objectives, test questions, and application form.

From the laboratory point of view, test results are only as reliable as the quality of the sample. It is imperative that the specimens received for coagulation testing are collected by a clean venipuncture with as little trauma to the tissues as possible. Clotted, hemolyzed, and contaminated specimens are not suitable and should not be used by the laboratory. The most common causes of erroneous results are related to the blood draw; therefore, special care must be taken to avoid any undue problems. Sodium citrate is the anticoagulant that is most commonly used for both routine and special coagulation testing. The citrated blood should be spun by routine centrifugation methods so that platelet free plasma is obtained.

PROTHROMBIN TIME
The prothrombin time (PT) monitors the factors that are in the extrinsic pathway – factors VII, X, V, and II. A PT is performed by adding synthetically prepared tissue factor, generally from brain or lung, and calcium to the test plasma and measuring the time it takes the clot to form. Prolongation of the PT is most often a result of deficiencies in factor VII but can also be caused by any of the aforementioned factors. Decreased fibrinogen, levels less than 100 mg/dL, will also prolong the PT; however, this test is not used to measure fibrinogen deficiency since most coagulation labs can perform a fibrinogen assay.

ACTIVATED PARTIAL THROMBOPLASTIN TIME
The partial thromboplastin time reagent is a preparation of synthetic phospholipids, particulate activator, and calcium. The APTT, or simply PTT, measures the factors that are designated in the intrinsic pathway – PK, HMWK, factors XII, XI, IX, VIII, X, V, II, and fibrinogen. Deficiencies of PK, HMWK, and factor XII show no bleeding tendencies and are of little clinical consequence.

There are several explanations for a prolonged PTT. The first, and most common, is heparin. Heparin may be present as a result of therapy or as a contaminant when it is used to flush a line. If heparin is suspected, a thrombin clotting time (TCT) can be performed. In this test, thrombin is added to plasma, and the clotting time is measured. The reference range for the TCT is 15 to 22 seconds. If heparin is present, the TCT will generally be prolonged to greater than 60 seconds.

A second possibility for a prolonged PTT is a factor deficiency. Most factor deficiencies are congenital and cause bleeding problems in children. Adult onset factor deficiencies are rather rare but can be the cause of severe bleeding problems. Acquired multiple factor deficiencies will have a PT and PTT of greater than 1.5 times the mean of the reference interval. Some causes of acquired factor deficiencies include liver disease, vitamin K deficiency, and drug therapy. To distinguish between liver disease and vitamin K deficiency, it is best to use factor V and factor VII levels. In liver disease both factors will be decreased, but in Vitamin K deficiency only factor VII will be low.

Finally, the presence of an inhibitor can also cause an abnormal PTT result. Inhibitors can be non-specific such as a phospholipid dependent inhibitor like lupus anticoagulants,
or they can be directed against a specific factor. Specific factor inhibitors, such as VIII or IX, prolong the PTT by lowering the amount of factor present.

Mixing studies should be performed to differentiate between a factor deficiency and the presence of an inhibitor. In this test, pooled normal plasma is mixed with the patient’s plasma in equal volumes, and a PTT is run on the sample. If the result is within five seconds of the pooled normal plasma, the mix is said to have corrected, and a factor deficiency is suspected. If there is no correction, the possibility of an inhibitor should be investigated.\(^1\)

**PLATELET COUNT**

At any age, a normal platelet count is 150 x 10\(^9\)/L to 450 x 10\(^9\)/L. Whenever the platelet count drops below 150 x 10\(^9\)/L, termed thrombocytopenia, it should be confirmed by examining a blood smear. The peripheral blood smear is important because the automated cell counters that are used in most laboratories can over or underestimate the platelet count. Refer to Table 1 for a list of possible causes of interference with the automated platelet count.

**DISSEMINATED INTRAVASCULAR COAGULATION**

Disseminated intravascular coagulation (DIC) is a condition in which there is a generalized activation of hemostasis. In this condition, coagulation factors and control proteins, platelets, and fibrinolytic enzymes are consumed; even though this is a thrombotic process, hemorrhage is often the most obvious symptom.\(^2\) The diagnosis of DIC must be confirmed quickly since acute DIC can be fatal. Clinically, however, DIC can present a very confusing picture. A laboratory panel of a platelet count, a PT, PTT, fibrinogen assay, and D-dimer assay is essential to aid the diagnosis. Expected results for a DIC panel can be found in Table 2.

The fibrinogen assay is similar to the TCT. In this test, thrombin is added to the sample plasma and acts as a catalyst to convert fibrinogen to fibrin. The two tests differ in that the fibrinogen assay uses diluted plasma at a 1:10 with Owren’s buffer, and the thrombin reagent used is at a higher concentration than that used in the TCT assay. These two variations give an “inverse but linear relationship between interval to clot formation and concentration of functional fibrinogen”.

The D-dimer assay is essential to the diagnosis of DIC. D-dimers are found in almost all cases of DIC; however, since D-dimer levels can be elevated in other conditions, it is imperative that the other tests in the DIC panel be used in conjunction with the D-dimer assay to reach a diagnosis.

Currently, there are two methods of testing D-dimers. The first is a semi-quantitative method in which latex particles are coated with monoclonal antibodies to D-dimers. Serial dilutions are made of the sample plasma and then mixed with the latex suspension. The test is read manually, and a positive result is determined if agglutination of the latex particles is observed. DIC is indicated by a positive reaction in the 1:2 dilution which suggests D-dimer levels of greater than 500 ng/mL.

The quantitative D-dimer assay is either an enzyme immunoassay or microlatex particle immunoagglutination assay method. The latter test can be automated and produces a result with a relatively rapid turn-around time. The quantitative D-dimer assay is most useful for its negative predictive value to aid in ruling out localized venous thrombosis such as pulmonary emboli and deep vein thrombosis. A normal, healthy adult can be

---

**Table 1. Possible interference with automated platelet counts**

<table>
<thead>
<tr>
<th>False increase</th>
<th>False decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microspherocytes</td>
<td>Poor collection techniques</td>
</tr>
<tr>
<td>Red blood cell and leukocyte fragments</td>
<td>EDTA–dependent platelet agglutination</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Platelet cold agglutinins</td>
</tr>
<tr>
<td>Pappenheimer bodies</td>
<td>Platelet satellitism</td>
</tr>
</tbody>
</table>

**Table 2. Expected results for a DIC profile**

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal range</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>150 – 450 x 10(^9)/L</td>
<td>&lt;150 x 10(^9)/L</td>
</tr>
<tr>
<td>PT</td>
<td>11 – 14 seconds</td>
<td>&gt;14 seconds</td>
</tr>
<tr>
<td>PTT</td>
<td>25 – 35 seconds</td>
<td>&gt;35 seconds</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>200 – 400 mg/ dL</td>
<td>&lt;200 mg/dL</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>0 – 240 ng/ mL</td>
<td>&gt;500 ng/mL</td>
</tr>
</tbody>
</table>
expected to have a D-dimer of less than 240 ng/mL. Higher levels are expected in patients with clots but are also seen in many other diseases. Clinical history and the laboratory tests previously described will aid the physician in reaching the correct diagnosis. Table 3 shows the correlation between the semi-quantitative and quantitative D-dimer assays.³

**FACTOR ASSAYS**

Hemophilia A, or factor VIII deficiency, is the most commonly inherited defect of the coagulation factors. The disease is an X-linked chromosome disorder; so therefore, it is primarily expressed in the male population. Laboratory screening tests show a normal PT and TCT. The PTT will generally be prolonged, but only if the factor VIII activity level is below 40%.

The factors that are necessary for clotting in the intrinsic pathway can each be measured by one-stage clotting assays as a modified version of the PTT. The specific factor assays measure the time of clot formation of test plasma when diluted and mixed with specific factor deficient substrate plasma. The result is compared to a corresponding calibration curve specific to the lot of PTT reagent and factor deficient plasma. Specimens are tested at three dilutions. The dilutions need to agree within 10% to assure that no inhibitor is present. A hemophiliac is considered a severe case if the factor VIII level is less than 1%. A factor VIII level of 1% to 5% is called a moderated hemophiliac, and a mild deficiency is diagnosed if the patient has factor VIII levels between 5% to 25%.

Chromogenic factor VIII assays are also available to quantify the factor VIII level. New recombinant, B-domainless FVIII products that make it necessary to be able to accurately measure activity levels are now on the market. Discrepancies are reported between the chromogenic and clot based methods. Clot-based methods recover up to 50% lower activity for these products than the chromogenic assays which can have significant clinical implications for the management of patients with hemophilia A.

Like hemophilia A, hemophilia B, or factor IX deficiency (also known as Christmas disease), is an X-linked chromosome bleeding disorder. The diagnosis is reached by similar methods as hemophilia A. The PT and TCT will be normal, but the PTT may or may not be abnormal, depending on the sensitivity of the reagent being used. Based on laboratory tests and clinical history, a diagnosis can be determined by performing a one-stage clotting assay using factor IX deficient substrate plasma. As with the factor VIII assay, three dilutions are performed which must agree within 10%, to obtain a factor level of the test plasma.⁴

**von WILLEBRAND DISEASE**

von Willebrand disease (vWD) is the most common hereditary bleeding disorder affecting up to 1% to 2% of the general population. It is a condition in which there is a either a quantitative or qualitative defect in the von Willebrand factor (vWF), a protein that is necessary to platelet adhesion and acts as a carrier protein for factor VIII. Testing for vWD in the laboratory starts with a panel of tests consisting of a factor VIII activity level, a ristocetin cofactor (also known as vWF activity level), and a von Willebrand antigen level. It is important to note that the vWF level varies significantly according to blood groups. Table 4 shows the mean vWF activity associated with the corresponding blood group. In addition to variation in blood types, vWF can also be increased during pregnancy, hemorrhage, acute infection, and strenuous exercise. As a result, patients with a suspicious clinical history should be confirmed with subsequent testing over a period of time.⁵

The ristocetin cofactor activity, or vWF activity, is determined by the agglutination of standardized platelets in the presence of vWF using ristocetin. The platelets play a passive role in the procedure, but it is necessary that the ristocetin dependent receptor be intact. The vWF activity is the most useful assay to diagnose vWD. In this procedure, platelets are treated with ristocetin in the presence of dilutions of standard plasma using a platelet aggregometer. The ristocetin cofactor activity is proportional to the slope of the platelet agglutination curve.

**FOCUS: HEMORRHAGIC ABNORMALITIES**

<table>
<thead>
<tr>
<th>Slide latex endpoint dilution</th>
<th>Quantitative D-dimer in ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Less than 240</td>
</tr>
<tr>
<td>Undiluted</td>
<td>240 – 500</td>
</tr>
<tr>
<td>1:2</td>
<td>500 – 1000</td>
</tr>
<tr>
<td>1:4</td>
<td>1000 – 2000</td>
</tr>
<tr>
<td>1:8</td>
<td>2000 – 4000</td>
</tr>
<tr>
<td>1:16</td>
<td>4000 – 8000</td>
</tr>
<tr>
<td>1:32</td>
<td>8000 – 16,000</td>
</tr>
<tr>
<td>1:64</td>
<td>16,000 – 32,000</td>
</tr>
</tbody>
</table>
Once a standard curve is prepared, patient plasma is the source of vWF, an agglutination pattern can be discerned, and the vWF activity is determined from the standard curve.

The vWF antigen can be determined two ways. The first is the ELISA method. A plate is coated with rabbit anti-human vWF antibodies that bind the vWF to be measured. In the next step, anti-vWF antibodies coupled with peroxidase bind to the rest of the free antigenic determinants of vWF. The bound enzyme peroxidase is revealed by its activity in a predetermined time on the substrate OPD (orthophenylenediamine) in the presence of hydrogen peroxide (H$_2$O$_2$). After the reaction is stopped with sulfuric acid (H$_2$SO$_4$), the color intensity is directly proportional to the concentration of vWF present. Absorbance is measured at a wavelength of 492 nm. The disadvantages to this test are that it is very time consuming and highly labor intensive.

Another option to determine the vWF antigen level is the immunoturbidimetric method. This is an automated assay in which a beam of light is passed through the sample. The sample consists of patient plasma added to a suspension of microlatex particles that have antibodies attached to them. If the wavelength of light is greater than the diameter of the particles, the light will only be slightly absorbed. When there is vWF antigen present, the particles will clump to form aggregates whose diameters are larger than the wavelength of light; therefore, more light is absorbed. This greater absorption is proportional to the vWF antigen level that is in the sample. Table 5 shows the various subtypes of von Willebrand disease, and the profile results expected.

Once a diagnosis of von Willebrand disease is made, it is sometimes necessary to order more testing to differentiate between the various subtypes. Assays such as the ristocetin response curve (also called low dose ristocetin induced platelet aggregation) will aid in distinguishing type 2B vWD. Platelets in Type 2B vWD are hyper-responsive to low concentrations of ristocetin. In addition, vWF multimeric analysis by SDS polyacrilamide gel electrophoresis can distinguish between Type 1 and 2 and also Subtypes 2A and 2B.

Diagnosis of the bleeding patient can be a difficult task. Clinical history is vital, but laboratory testing provides key information into the patient’s condition. Proper use of this information can save valuable time when the need to assess the situation quickly is critical.6

REFERENCES

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Mean vWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>75%</td>
</tr>
<tr>
<td>A</td>
<td>105%</td>
</tr>
<tr>
<td>B</td>
<td>117%</td>
</tr>
<tr>
<td>AB</td>
<td>123%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>vWD type</th>
<th>vWF activity</th>
<th>vWF antigen activity</th>
<th>Factor VIII activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 – 60%</td>
<td>40 – 60%</td>
<td>40 – 60%</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>2A</td>
<td>30 – 50%</td>
<td>70 – 150%</td>
<td>70 – 150%</td>
</tr>
<tr>
<td>2B</td>
<td>30 – 50%</td>
<td>70 – 150%</td>
<td>70 – 150%</td>
</tr>
<tr>
<td>2N</td>
<td>70 – 150%</td>
<td>40 – 60%</td>
<td>70 – 150%</td>
</tr>
<tr>
<td>2M</td>
<td>40 – 60%</td>
<td>40 – 60%</td>
<td>70 – 150%</td>
</tr>
</tbody>
</table>
FOCUS: HEMORRHAGIC ABNORMALITIES

Use of Blood Products and Factor Concentrates for Coagulation Therapy

MARGARET G FRITSMA

ABBREVIATIONS: APTT = activated partial thromboplastin time; DIC = disseminated intravascular coagulation; FFP = fresh frozen plasma; HIT = heparin-induced thrombocytopenia; ITP = idiopathic thrombocytopenic purpura; PT = prothrombin time; TTP = thrombotic thrombocytopenic purpura.

INDEX TERMS: Activated prothrombin complex concentrate; cryoprecipitate; factor replacement concentrates; fresh frozen plasma; platelet concentrate.

Clin Lab Sci 2003;16(2):115

Margaret G Fritsma MA MT(ASCP) SBB is Associate Professor, University of Alabama at Birmingham, Birmingham AL.

Address for correspondence: Margaret G Fritsma MA MT (ASCP) SBB, Associate Professor, School of Health Related Professions, Clinical Laboratory Sciences, 1705 University Boulevard, RMSB Room 450, Birmingham AL 35294-1212. (205) 934-5987. (205) 975-7302 (fax). fritsmam@uab.edu

George A Fritsma is the Focus: Hemorrhagic Abnormalities guest editor.

Focus Continuing Education Credit: see pages 123 to 126 for learning objectives, test questions, and application form.

Appropriate replacement therapy can be life-saving for patients with hemorrhage due to coagulation disorders. Effective treatment depends on accurate diagnosis of the hemostatic defect, choosing the appropriate therapeutic agent, and monitoring the patient’s clinical and laboratory response. A variety of traditional blood components and newer factor concentrates are available, each with its own advantages and limitations for use. Indiscriminate use of blood products should be avoided, as it is costly, wastes resources, subjects the patient to unnecessary risks, and produces limited or no clinical benefit.

BLOOD COMPONENTS

Fresh frozen plasma

Fresh frozen plasma (FFP) is the plasma from a unit of whole blood separated by centrifugation and frozen within eight hours of collection from the donor. It is stored at –18 °C or lower for up to 12 months, thawed at 30 to 37 °C and kept at 1 to 6 °C for no longer than 24 hours. FFP contains an average of 1 IU/mL of all the coagulation proteins, including the labile factors V and VIII. (An IU is defined as the amount of coagulation factor present in one mL of normal plasma.)

FFP is primarily used to treat bleeding due to acquired multiple factor deficiencies that occur in liver disease, vitamin K deficiency, disseminated intravascular coagulation (DIC), and massive transfusion. Less frequently, it may be used to treat the rare congenital single factor deficiencies of II, V, VII, X, or XI, or deficiencies of protein C or S. (Because of its short half-life of three to six hours, factor VII deficiency is difficult to treat with FFP without volume overload.) FFP may be used for immediate short-term reversal of over-anticoagulation with coumadin. Both FFP and cryoprecipitate reduced plasma are used as replacement components in therapeutic plasma exchange for thrombotic thrombocytopenic purpura (TTP).

A dose of 10 to 20 mL of FFP/kg of body weight will usually increase the factor level by 20% to 30%. Frequency of transfusion depends on the half-life of the deficient factor(s), shown in Table 1.

The use of FFP is not indicated unless the prothrombin time (PT) or activated partial thromboplastin time (APTT) is >1.5 times the mean of the normal range. FFP should not be used as a volume expander, or to ‘correct’ a mildly prolonged PT or APTT. A patient may have a mildly prolonged PT or APTT and yet have hemostatically stable levels of coagulation factors.
Cryoprecipitate

Cryoprecipitate is the protein precipitate left after FFP is thawed at 4 °C and most of the supernatant liquid plasma removed. Cryoprecipitate is refrozen and stored at −18 °C or lower for up to 12 months. After thawing at 30 to 37 °C, it is kept at 20 to 24 °C for no longer than six hours, or if pooled, no longer than four hours. It contains a minimum of 80 IU of factor VIII, vWF, 150 to 250 mg of fibrinogen, and 50 to 75 IU of factor XIII.2,3

Cryoprecipitate is most commonly infused to replace fibrinogen, for either acquired deficiencies due to DIC or thrombolytic therapy, or for congenital hypofibrinogenemia or dysfibrinogenemias. Currently, cryoprecipitate is the only source of concentrated fibrinogen available. A fibrinogen level of 50 to 100 mg/dL is considered hemostatically effective, and can be achieved using a general guideline of infusing one bag cryo/seven kg of body weight.4 Fibrinogen has a half-life of 100 to 150 hours.1

Cryoprecipitate is also used to treat the rare congenital or acquired deficiency of factor XIII. Factor XIII has a long half-life, seven to twelve days, so the recommended treatment for factor XIII deficiency is one bag of cryo/ten kg of body weight every seven days.5

Although factor VIII and vWF are present in cryoprecipitate, cryo is no longer recommended for replacement therapy for hemophilia A or von Willebrand’s disease because of the risk of disease transmission. Recombinant or virally inactivated FVIII concentrates are the preferred treatment.

Platelets

Two types of platelet components are used for platelet replacement therapy:

- ‘Platelets’, also called random donor platelets, are pooled concentrates prepared from whole blood donations by centrifugation. Each unit in the pool should have a minimum of 5.5 x 10¹¹ platelets. Usually a pool consists of four to eight units, for an adult dose of one unit/ten kg of body weight.6
- ‘Platelets Pheresis’, or single donor platelets, are prepared by apheresis of one donor, and contain a minimum of 3 x 10¹¹ platelets.6

Both random donor platelets and apheresis platelets are stored for five days at 20 to 24 °C with constant agitation.

Therapeutic platelet transfusions are given to treat bleeding due to defects in platelet production or platelet function. Transfusion of platelets is contraindicated for diseases in-

<table>
<thead>
<tr>
<th>Table 1. Coagulation factor replacement therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting factor</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Fibrinogen (Factor I)</td>
</tr>
<tr>
<td>Factor II</td>
</tr>
<tr>
<td>Factor V</td>
</tr>
<tr>
<td>Factor VII</td>
</tr>
<tr>
<td>Factor FVIII</td>
</tr>
<tr>
<td>Factor IX</td>
</tr>
<tr>
<td>Factor X</td>
</tr>
<tr>
<td>Factor XI</td>
</tr>
<tr>
<td>Factor XIII</td>
</tr>
<tr>
<td>FVIII with inhibitor</td>
</tr>
<tr>
<td>FIX with inhibitor</td>
</tr>
<tr>
<td>VonWillebrand factor</td>
</tr>
</tbody>
</table>
volving thrombotic consumption of platelets, including thrombotic thrombocytopenic purpura (TTP), heparin-induced thrombocytopenia (HIT), and active DIC. Platelet transfusions are not usually effective in immune-mediated thrombocytopenias, such as idiopathic thrombocytopenic purpura (ITP), although there may be some benefit in life-threatening situations.

Indications for prophylactic platelet transfusions are less definitive. It is difficult to establish a specific platelet count threshold for prophylactic transfusion since the patient’s clinical condition, and the risk, presence, and cause of bleeding are individualized. Complicating factors affecting the response to platelet transfusion include fever, sepsis, consumption, bleeding, ABO incompatibility between patient and platelets, splenomegaly, platelet count of the donor(s), medications, presence of other hemostatic defects, and immune refractoriness. Nevertheless, a threshold of 10 x 10^9/L to 20 x 10^9/L is commonly used as an indication for prophylactic platelet transfusion.

Ideally, effectiveness of platelet transfusions will be shown by cessation of bleeding. If there are no complicating factors affecting response as listed above, a unit of platelets should increase the recipient’s platelet count by 5 x 10^9/L to 10 x 10^9/L, and a unit of ‘Platelets Pheresis’ should increase the count by 30 x 10^9/L to 50 x 10^9/L. Many multi-transfused patients do not show the expected increment response to platelet transfusion. The most common method to determine if the recipient is refractory to platelet transfusion is to measure the platelet count within one hour posttransfusion and calculate the corrected count increment (CCI), shown below:

\[ \text{CCI} = \frac{(\text{post transfusion platelet count} - \text{pretransfusion platelet count}) \times \text{body surface area (m}^2) \div \text{number of platelets transfused (multiples of 10}^{11})}{\text{number of platelets transfused (multiples of 10}^{11})} \]

A CCI above 7500 indicates an adequate response to platelet transfusion.

For patients who are immunized to HLA antigens and are refractory to platelet transfusion, HLA-matched or crossmatch-compatible platelets may be effective. These units should be irradiated to prevent transfusion-associated graft-versus-host disease.

Platelet refractoriness in multi-transfused patients has been shown to be related to the presence of Class II HLA antigens on contaminating leukocytes, rather than to the number of donor exposures. Therefore, leukocyte-reduced platelets are recommended to reduce the incidence of HLA immunization in patients receiving frequent platelet transfusions.

**PLASMA AND NON-PLASMA FACTOR CONCENTRATES**

A variety of factor concentrates are available to treat hemophilia A (FVIII deficiency), hemophilia B (FIX deficiency), and von Willebrand’s disease (vWF deficiency), as described below.

**Plasma derived factor concentrates**

These concentrates (Table 2) are prepared by fractionation of large pools of thousands of units of donor plasma. They are processed to inactivate contaminating viruses, using pasteurization techniques, solvent/detergent treatment, or immunoaffinity column purification. Although they are considered quite safe, they are not totally risk free, in that they could potentially transmit nonlipid viruses such as hepatitis A and human parvovirus 19.

**Intermediate purity FVIII**

Humate-P® and Alphanate® contain factor VIII and von Willebrand factor. Humate-P® is licensed to treat both hemophilia A and von Willebrand’s disease. Clinical trials using Alphanate® for treatment of von Willebrand’s disease have not yet been done; therefore Alphanate® is only approved for treatment of hemophilia A.

**Immunoaffinity high purity FVIII concentrate**

These products are prepared by monoclonal antibody column purification and do not contain vWF. Examples of licensed products are Monarch-M®, Hemofil-M®, Monoclate®, and Koate-HP®.

**Porcine FVIII**

Hemophiliacs who have produced inhibitors against human FVIII may be successfully treated with porcine FVIII. Hyate-C® is the licensed product and the recommended initial dose is 100 U FVIII/t en kg of body weight.

**Immunoaffinity High Purity FIX Concentrate**

These FIX concentrates are prepared using monoclonal antibody affinity columns. Examples are AlphaNine SD® and Mononine®.

**Prothrombin complex concentrate (PCC)**

Prothrombin complex concentrates consist of factors II, IX, and X. Factor VII may also be present depending on the method of production. PCCs may produce allergic or thrombotic side effects, and are indicated for treatment of rare de-
ficiencies of II, VII, or X. They may also be beneficial in treatment of hemophiliacs with factor inhibitors. They are not recommended for treatment of FIX deficiency. Examples of licensed products are Proplex T™ (contains FVII), Konyne®, Profilnine HT®, and Bebulin®.3,6

Activated prothrombin complex concentrates (APCC)
Activated complex products are prothrombin complex concentrates in which the coagulation proteins are activated. They are used to treat hemophiliacs with inhibitors by bypassing FVIII or FIX activation. The recommended dose is 50 to 100 IU/kg. Repeat doses may be given at 6-, 8-, 12- or 24-hour intervals depending on the extent of the bleeding. To avoid thrombotic complications, the amount given should not exceed 200 IU/kg/24 hours. Examples of APCCs are FEIBA® and Autoplex®.3,5

Non-plasma recombinant factor concentrates
Factor concentrates produced by recombinant DNA technology (Table 3) have the highest purity and level of safety but are more costly than virally inactivated plasma products. Because they are not produced from plasma they do not transmit diseases.

FVIII concentrate
Examples are Recombinate®, Kogenate®, Biocluate®, Helixate®, and ReFacto®.3,6

FIX concentrate
BeneFIX® is the licensed recombinant FIX product.3

Factor VIII replacement
To calculate the initial or loading dose of factor VIII:

\[
\text{Blood volume} = \text{weight (kg)} \times 70 \text{ mL/kg}
\]

\[
\text{Plasma volume} = \text{blood volume} \times (1.0 - \text{hematocrit})
\]

\[
\text{Required Units of FVIII} = (\text{desired level} - \text{initial level}) \times \text{X plasma volume}
\]

The maintenance dose is 50% of the initial dose, and is administered every 12 hours, since the half-life of FVIII is 12 hours.

Table 2. Plasma derived coagulation factor concentrates

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Deficiency/Therapeutic Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate purity FVIII</td>
<td>Humate-P Alphanate</td>
<td>FVIII and vWF FVIII</td>
</tr>
<tr>
<td>High purity FVIII</td>
<td>Monarch-M Hemofil-M Monoclate Koate-HP</td>
<td>FVIII FVIII FVIII</td>
</tr>
<tr>
<td>Porcine FVIII</td>
<td>Hyate-C</td>
<td>FVIII w/inhibitor</td>
</tr>
<tr>
<td>High purity FIX</td>
<td>AlphaNine SD Mononine</td>
<td>FIX FIX</td>
</tr>
<tr>
<td>Prothrombin complex concentrate (PCC)</td>
<td>Proplex Konyne Profilnine-HT Bebulin</td>
<td>FVIII or FIX w/inhibitor, FII, FVII, and FX FVIII or FIX w/inhibitor, FII, and FX FVIII or FIX w/inhibitor, FII, and FX FVIII or FIX w/inhibitor FVIII or FIX w/inhibitor</td>
</tr>
<tr>
<td>Activated prothrombin complex concentrate (APCC)</td>
<td>FEIBA Autoplex</td>
<td>FVIII or FIX w/inhibitor FVIII or FIX w/inhibitor</td>
</tr>
</tbody>
</table>
FOCUS: HEMORRHAGIC ABNORMALITIES

Factor IX replacement
Therapy for FIX deficiency is determined using the same formula as FVIII, except that the calculated dose is doubled since factor IX diffuses into the extravascular fluid. The half-life of FIX is 18 to 24 hours, so repeat doses of half of the initial loading dose are administered once daily.

vWF replacement
For treatment of von Willebrand’s disease, the usual therapeutic target is 50%, or 0.5 IU, of vWF. The dosage is calculated using the same formula for FVIII shown above. The maintenance dose is 50% of the initial dose, and is administered every 12 hours, since the half-life of vWF is 12 hours.

SUMMARY
Therapy of coagulation disorders has evolved from early use of fresh whole blood and plasma, to sophisticated recombinant factor concentrates. Although current testing protocols and viral inactivation methods ensure that transfusion of components is safer than ever, the potential for new threats continually exists, e.g., West Nile virus.

Effective therapy depends on treating the specific deficiency with the safest and most appropriate replacement product, in the proper dose.

REFERENCES

<p>| Table 3. Non-plasma coagulation factor concentrates |</p>
<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Deficiency/Therapeutic Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant FVIII</td>
<td>Recombinate</td>
<td>FVIII</td>
</tr>
<tr>
<td></td>
<td>Kogenate</td>
<td>FVIII</td>
</tr>
<tr>
<td></td>
<td>Bioclate</td>
<td>FVIII</td>
</tr>
<tr>
<td></td>
<td>Helixate</td>
<td>FVIII</td>
</tr>
<tr>
<td></td>
<td>ReFacto</td>
<td>FVIII</td>
</tr>
<tr>
<td>Recombinant FIX</td>
<td>BeneFIX</td>
<td>FIX</td>
</tr>
<tr>
<td>Recombinant VIIa</td>
<td>NovoSeven</td>
<td>FVIII or FIX with inhibitor</td>
</tr>
</tbody>
</table>

<p>| Table 4. Guidelines for hemophilia treatment by bleeding site |</p>
<table>
<thead>
<tr>
<th>Site of Bleed</th>
<th>Desired Factor VIII or IX activity</th>
<th>Days Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial; trauma with bleeding</td>
<td>100%</td>
<td>10-14</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>80-100%</td>
<td>1-3</td>
</tr>
<tr>
<td>Major surgery</td>
<td>80-100%</td>
<td>5-14 days post-op</td>
</tr>
<tr>
<td>Hematuria</td>
<td>50%</td>
<td>1-3</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>50%</td>
<td>1-3</td>
</tr>
<tr>
<td>Single joint</td>
<td>30-50%</td>
<td>1-3</td>
</tr>
<tr>
<td>Minor surgery</td>
<td>30% to 3-4 days post-op</td>
<td></td>
</tr>
<tr>
<td>Mucosal, severe epistaxis</td>
<td>30%</td>
<td>1-2</td>
</tr>
</tbody>
</table>
FOCUS: HEMORRHAGIC ABNORMALITIES

Treatment of Single Factor Deficiencies: A Case Study Approach

MARISA B MARQUES

INDEX TERMS: Factor VIII concentrate; factor VIII inhibitor; hemophilia; von Willebrand disease.

Clin Lab Sci 2003;16(2):120

Marisa B Marques MD is the coagulation service medical director at the University of Alabama at Birmingham, Birmingham AL.

Address for correspondence: Marisa B Marques MD, Department of Pathology, University of Alabama at Birmingham, 619 South 19th Street, West Pavilion, P230, Birmingham AL 35293. http://uabscoag.net

George A Fritsma is the Focus: Hemorrhagic Abnormalities guest editor.

Focus Continuing Education Credit: see pages 123 to 126 for learning objectives, test questions, and application form.

von Willebrand disease and hemophilia A are the two most common inherited bleeding disorders.1 The diagnosis, treatment, and monitoring of patients with these conditions requires frequent interaction between the patient’s physician and the clinical laboratory. In many institutions, coagulation factor concentrates are dispensed from the blood bank along with other blood-derived products, while the special coagulation laboratory offers assays to monitor response to treatment. It is important in these instances that there is open communication among the staff of those laboratories and an understanding of the important issues in the management of patients with von Willebrand disease or hemophilia A with or without inhibitor.2 In this discussion, such issues are highlighted using a case study-based approach.

CASE 1: von WILLEBRAND DISEASE

A 40-year old white female was hospitalized to have left total knee arthroplasty on the next day. Her past medical history was significant for severe von Willebrand disease and multiple episodes of hemorrhosis in the affected joint. The orthopedic surgeon called the coagulation consultant to decide on von Willebrand factor replacement for surgery.

Although von Willebrand disease is classically associated with manifestations of abnormal primary hemostasis such as mucosal bleeding, in the severe form of this disease, patients also have a deficiency of factor VIII and suffer from joint bleeds. The deficiency of factor VIII is a consequence of increased proteolysis of this factor in the absence of normal levels of von Willebrand factor to protect it in the circulation. Until a few years ago, cryoprecipitate was the product of choice to treat patients with severe von Willebrand disease. However, plasma-derived concentrates of von Willebrand factor are now available and have become the first choice for treatment of this condition. Since von Willebrand factor and factor VIII circulate together, these replacement products contain both factors. The only Food and Drug Administration (FDA) approved product is Humate-P® which contains approximately 2.5 times more von Willebrand factor than factor VIII. In order to calculate the appropriate dose of factor to prepare the patient for surgery, several patient-specific pieces of information are necessary (Table 1). The data for this patient were:

- Baseline von Willebrand factor level: <1% or <0.01 units/mL of plasma
- Baseline factor VIII level: 1% or 0.01 units/mL of plasma
- Target von Willebrand factor level: 90% to 100% or 0.9 to 1 unit/mL of plasma (for major surgery)
- Weight: 107 lb or 48.5 Kg (1 lb = 0.453 Kg)
- Hematocrit (HCT): 25%
- Blood volume (BV): weight (48.5 Kg) x 70 mL/Kg (for patients with body mass index (BMI) < 25; multiply by 60 mL/Kg if BMI between 25 and 30; and by 50 mL/Kg if BMI > 30) = 3,395 mL
- Plasma volume (PV): BV x (1 – HCT) or 3,395 x 0.75 = 2,546 mL
- Dose in units: (desired activity [0.9 to 1 unit/mL] - baseline activity [zero] ) x PV = 2,291 to 2,546 units

Thus, in order to increase the von Willebrand factor level from zero to close to 100%, she should receive the appropriate number of vials of Humate-P that more closely add to the calculated dose of 2546 units. Since each lot of coagulation factor concentrates contain different amounts of each factor, the person dispensing the dose should calculate how...
many vials are necessary to fulfill the order. To be certain that the calculated dose is correct, a factor level can be measured 1 hour after the infusion is complete. In this patient, the von Willebrand factor (ristocetin cofactor activity) and factor VIII levels were 90% and 73%, respectively. Since the half-life of von Willebrand factor is approximately 12 hours, the following doses for maintenance of factor levels for surgery and the post-operative period should be half of the loading dose (approximately 1,250 units) repeated every 12 hours. Even when the patient is stable and not bleeding, it is necessary to follow von Willebrand factor levels once a day while in the hospital. It is also important to remember that if subsequent factor VIII levels are measured in these patients, they will be higher than expected based on the amount of factor VIII given. The reason for this discrepancy is that once von Willebrand factor is provided, the factor VIII level will reflect the added effect of what was infused through Humate-P plus the endogenous production of factor VIII which now resists proteolysis in plasma. Finally, factor replacement needs to be continued at home for seven to ten days post-operatively and the patient needs to seek immediate medical attention if abnormal bleeding ensues.

CASE 2: HEMOPHILIA A
A 35-year old white male with mild hemophilia A was admitted for persistent bleeding from a skin cut incurred by a car fender several hours earlier. His past medical history was significant for appendectomy, which resulted in prolonged bleeding. The hematologist ordered replacement therapy after seeing the patient and reviewing the history and laboratory data. In treating patients with hemophilia A, the degree of factor VIII deficiency (mild, moderate, or severe) will affect treatment. Many patients with mild hemophilia A can avoid factor replacement by relying on DDAVP (1-deamino-8-D-arginine-vasopressin), which induces the release of factor VIII/von Willebrand factor from endothelial cells and platelets. If factor concentrates are indicated by the physician, the two main sources of factor VIII are plasma or recombinant technology. The factors purified from plasma undergo viral inactivation steps to decrease the risk of transmitting infection by lipid-enveloped viruses such as hepatitis B and C and human immunodeficiency virus (HIV). These high-purity factor VIII products (Table 2) are the first line therapy for patients who have received plasma products before or are already infected with one or more of these viruses. Newly diagnosed patients, those never treated with plasma-derived factor previously or those whose previous treatment is unknown, should receive factor VIII produced by recombinant technology in tissue culture (Table 2). Unfortunately, these factors are sometimes in short supply and not readily available. For those instances, one needs to realize that the plasma-derived products have never been shown to transmit hepatitis or HIV, thus constituting a very safe alternative.

The data for the calculation of the factor VIII dose for this patient were as follows:
- Baseline factor VIII level: 19% or 0.19 units/mL of plasma (mild hemophiliac)
- Factor VIII inhibitor assay - negative
- Serology for hepatitis C - positive

<table>
<thead>
<tr>
<th>Table 1. Important patient data to calculate coagulation factor replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient factor, i.e., von Willebrand factor, Factor VIII, Factor IX</td>
</tr>
<tr>
<td>Presence or absence of factor inhibitor</td>
</tr>
<tr>
<td>Baseline factor level</td>
</tr>
<tr>
<td>Reason for replacement to establish target level</td>
</tr>
<tr>
<td>Weight in Kg</td>
</tr>
<tr>
<td>Hematocrit</td>
</tr>
<tr>
<td>Calculated blood and plasma volumes</td>
</tr>
<tr>
<td>Half-life of factor to decide on dosage interval</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Coagulation factor products to treat Hemophilia A</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-purity plasma-derived factor VIII products</td>
</tr>
<tr>
<td>Hemophil-M</td>
</tr>
<tr>
<td>Koate-HP</td>
</tr>
<tr>
<td>Monarch-M</td>
</tr>
<tr>
<td>Monoclate-P</td>
</tr>
<tr>
<td>Factor VIII prepared by recombinant technology</td>
</tr>
<tr>
<td>Bioclate</td>
</tr>
<tr>
<td>Helixate</td>
</tr>
<tr>
<td>Kogenate</td>
</tr>
<tr>
<td>Recombinate</td>
</tr>
<tr>
<td>Alternatives for patients with factor VIII inhibitors</td>
</tr>
<tr>
<td>Activated prothrombin complex concentrates:</td>
</tr>
<tr>
<td>Autoplex T</td>
</tr>
<tr>
<td>FEIBA</td>
</tr>
<tr>
<td>Recombinant factor VIIa (NovoSeven)</td>
</tr>
<tr>
<td>Porcine factor VIII (Hyate C)</td>
</tr>
</tbody>
</table>
FOCUS: HEMORRHAGIC ABNORMALITIES

- Target factor VIII level: 75% or 0.75 units/mL of plasma
- Weight: 160 lb or 72.5 Kg (1 lb = 0.453 Kg)
- Hematocrit (HCT): 35%
- Blood volume (BV): weight (72.5 Kg) x 70 mL/Kg = 5,100 mL
- Plasma volume (PV): BV x (1 – HCT) or 5,100 x 0.65 = 3,315 mL
- Dose in units: (desired activity [0.75 units/mL] - baseline activity [0.19 units/mL]) x PV = 1,856 units of plasma-derived factor VIII (loading dose).

Since the factor VIII circulating half-life is between eight to 12 hours, the patient should receive approximately 900 units of factor VIII two or three times daily depending on the severity of the bleeding, the clinical response and the factor VIII levels measured throughout treatment.

CASE 3: HEMOPHILIA A WITH INHIBITOR
A 47-year old male with hemophilia A presented to the emergency department complaining of left knee swelling. The patient reported that he has a factor VIII inhibitor and cannot receive factor VIII concentrates to stop the hemorrhage. The coagulation service was consulted. Review of the laboratory records confirmed that the patient suffers from severe hemophilia A and developed a factor VIII inhibitor since the first time he was tested at our hospital six years previously.

Approximately one third of patients with severe hemophilia A, those with <1% factor VIII activity, develop an IgG alloantibody directed to the factor after exposure to plasma-derived or recombinant factor VIII. The amount of inhibitor is quantitated by the Bethesda assay and the inhibitor is expressed in terms of Bethesda units (BU). One Bethesda unit is defined as the reciprocal of the patient’s plasma dilution that inactivates 50% of the amount of factor VIII in normal human plasma. When the patient has less than five to ten BU of inhibitor, he may be treated with enough factor VIII to overcome the presence of the inhibitor in the plasma and supply a hemostatic level. However, after further exposure to factor VIII, the level of inhibitor can increase significantly. In the case of this patient, for instance, his inhibitor was at 2 BU when he received factor VIII injections in the past. When he returned to the outpatient clinic two months later, his inhibitor level had increased to 225 BU. Since then, he has required an alternative treatment to stop bleeding or prepare him for a surgical procedure. Options for patients with inhibitors are described in Table 2. Since porcine factor VIII is not being produced, only the first two types of products are currently available in the United States. In our hospital we have used FEIBA as the first choice for many years.

The dose of FEIBA is calculated by multiplying the patient’s weight in Kg by 50 or 100 units and the dose interval varies from every six to every 12 hours with a maximum daily dose of 200 units/Kg per day. The total daily dose and the interval between injections depend on the severity of the bleed and the response to treatment. The doses are independent of the patient’s inhibitor level and there is no laboratory test to monitor therapy. Clinical response is the only parameter to be followed. The last statement also applies to NovoSeven, recombinant activated factor VII, which is the newest product to treat patients with factor VIII inhibitors. The dose of NovoSeven is 90-120 mg/Kg repeated every two to three hours until the bleeding has stopped.

CONCLUSION
The treatment of patients with single coagulation factor deficiencies has improved considerably in the last decade. Patients and physicians now count on a number of safe alternatives to control or prevent hemorrhage for the various disease states. It is imperative, however, that the proper diagnosis be available in order to choose the right product. Coordination between the laboratory and the physicians taking care of the patient will ensure the most efficient and cost-effective sequence of tests, factor injections, and therapy monitoring. In order to have clinical and laboratory expertise available, patients with von Willebrand disease and hemophilia are often referred to tertiary care centers instead of being managed in smaller facilities with fewer resources. A recent review of our records demonstrated that the average cost of treatment of these patients is a major portion of the Transfusion Medicine budget.

REFERENCES
FOCUS: HEMORRHAGIC ABNORMALITIES

Continuing Education Questions

SPRING 2003

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

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

LEARNING OBJECTIVES
The reader will be able to:
1. distinguish between anatomic and systemic hemorrhage.
2. distinguish between acquired and congenital hemorrhage.
3. select hemostatic laboratory tests that may be used to establish the presence of a hemostatic disorder.
4. identify hemostatic laboratory tests for use in pinpointing the cause of hemorrhage.
5. interpret the laboratory test profile results used to establish the presence of disseminated intravascular coagulation.
6. interpret the laboratory test profile results used to establish the presence of von Willebrand disease.
7. select single coagulation factor assays.
8. describe how to perform and interpret mixing studies.
9. determine the presence of a coagulation inhibitor.
10. select the appropriate coagulation factor concentrate therapy to treat hemorrhagic disorders.
11. calculate the correct dosage of coagulation factor concentrate to appropriately treat hemorrhagic disorders.
12. assess the dosage adequacy of the coagulation factor concentrate to appropriately treat hemorrhagic disorders.
13. detail the selection and dosage of factor concentrates for von Willebrand disease, hemophilia, and hemophilia with the presence of an inhibitor.

CONTINUING EDUCATION QUESTIONS
1. What is the most common acquired hemorrhagic disorder?
   a. Disseminated intravascular coagulation
   b. Vitamin K deficiency
   c. Liver disease
   d. Hemophilia

2. Both liver disease and vitamin K deficiency cause a prolonged PT and PTT. How do factor V and factor VII assays help determine which condition is present?
   a. FV is decreased in liver disease but not in vitamin K deficiency
   b. FVII is decreased in liver disease but not in vitamin K deficiency
   c. FV and FVII are decreased in both disorders
   d. FV is decreased in both disorders

3. A patient experiences petechiae and purpura on arms and legs, repeated nosebleeds, and hematemesis. What type of hemorrhage is present?
   a. Systemic hemorrhage
   b. Anatomic hemorrhage
   c. Local hemorrhage
   d. No hemorrhage

4. What factor deficiency is the prothrombin time most sensitive to?
   a. Prothrombin
   b. VII
   c. VIII
   d. IX

5. What condition causes a prolonged thrombin time test result?
   a. Prothrombin deficiency
   b. Antithrombin deficiency
   c. Presence of heparin
   d. Coumadin therapy

Sharon M Miller is the liaison for the CLS Continuing Education section. She reviews Focus articles, assigns contact hours, and edits learning objectives and test questions. Direct all continuing education inquiries to Sharon M Miller, 7N591 Cloverfield Circle, St Charles, IL 60175. (630) 513-1986. smmiller@elnet.com
6. If a patient has an anatomic bleeding disorder and poor wound healing but the PT, PTT, thrombin time, platelet count, and platelet functional assay results are all normal, what factor deficiency could exist?
   a. Fibrinogen
   b. Prothrombin
   c. Factor XII
   d. Factor XIII

7. The typical DIC test profile uses what tests?
   a. D-dimer, platelet count, fibrinogen assay, PT, and PTT
   b. Factor VIII assay, reptilase time, PT, thrombin time
   c. D-dimer, platelet aggregometry, fibrinogen assay, PT, and thrombin time
   d. Factor VII assay, Russell viper venom time, PTT, and thrombin time

8. What is the most likely interpretation of the following tests?

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>18 sec</td>
<td>11-14 sec</td>
</tr>
<tr>
<td>PTT</td>
<td>48 sec</td>
<td>25-35 sec</td>
</tr>
<tr>
<td>TCT</td>
<td>31 sec</td>
<td>15-22 sec</td>
</tr>
<tr>
<td>Platelet count</td>
<td>56 X 10^9/L</td>
<td>150-450 X 10^9/L</td>
</tr>
<tr>
<td>Semi quantitative</td>
<td>Pos 1:8</td>
<td>Negative</td>
</tr>
<tr>
<td>D-dimer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   a. Liver disease
   b. Vitamin K deficiency
   c. Thrombotic thrombocytopenic purpura (TTP)
   d. Disseminated intravascular coagulation (DIC)

9. The activated partial thromboplastin time (APTT, PTT) will be prolonged in each of the following EXCEPT a:
   a. FVII deficiency.
   b. FVIII deficiency.
   c. lupus anticoagulant.
   d. heparinized specimen.

10. A patient’s PTT was 62 seconds. After mixing the patient’s plasma with normal plasma and repeating the test, the PTT was 58 seconds. This indicates:
   a. a single factor deficiency.
   b. multiple factor deficiencies.
   c. the presence of inhibitor.
   d. that no inhibitor is present.

11. The test for von Willebrand factor (vWF) activity is:
   a. ELISA for vWF antigen.
   b. ristocetin cofactor.
   c. SDS-PAGE multimer assay.
   d. low-dose ristocetin induced platelet aggregation.

12. What is the plasma half-life of von Willebrand factor?
   a. Three to six hours
   b. 12 hours
   c. 24 hours
   d. Three to five days

13. What is the most commonly used treatment for acquired multiple factor deficiencies such as in liver disease?
   a. Cryoprecipitate
   b. Factor VIIa concentrate
   c. Factor VIII concentrate
   d. Fresh frozen plasma

14. What therapy might be used for a hemophilia patient who is bleeding but who has a low titer factor VIII inhibitor?
   a. Fresh frozen plasma
   b. Porcine factor VIII
   c. Antithrombin concentrate
   d. High dosage factor VIII concentrate

15. A patient with von Willebrand disease needs replacement therapy. Which product would be appropriate to use?
   a. Intermediate purity factor VIII concentrate (Humate P)
   b. Porcine FVIII (Hyate C)
   c. Recombinant FVIII (Recombinate)
   d. Cryoprecipitate

16. How many bags of cryoprecipitate should be given to obtain a hemostatic level of fibrinogen in a 64 kg patient with hypofibrinogenemia?
   a. Three
   b. Seven
   c. Nine
   d. Twelve
17. A bleeding patient with a platelet count of $7.0 \times 10^9$/L received one unit of 'Platelets Pheresis' containing $4.2 \times 10^{11}$/L platelets. The one-hour post-transfusion platelet count was $20 \times 10^9$/L. Is this an adequate response to transfusion? (The patient’s body surface area is 1.8 m$^2$.)
   a. Yes, a platelet count of $20 \times 10^9$/L protects against spontaneous hemorrhage.
   b. Yes, the CCI is 8000, which indicates an adequate response.
   c. No, the patient’s post-transfusion count should have been $100 \times 10^9$/L.
   d. No, with a CCI of 5600 the patient is likely refractory to platelet transfusion.

18. Fresh frozen plasma is indicated for transfusion in which of the following situations?
   a. Hemophilia A
   b. Hypofibrinogenemia
   c. Coagulopathy due to massive transfusion
   d. Prothrombin time = 16.0 seconds (mean of reference interval = 11.5 sec)

19. Of the following, which would be the safest choice to treat a bleeding patient who has hemophilia B?
   a. Konine
   b. BeneFIX
   c. Autoplex
   d. Monoclate

20. Which of the following prothrombin complex concentrates is appropriate for treatment of a patient with factor VII deficiency?
   a. Proplex
   b. Konyne
   c. Profilnine
   d. Bebulin

21. Calculate the dosage of FVIII needed to raise the FVIII activity of a 175 lb (80 kg) man from 2% to 80%. His hematocrit is 35%.
   a. 1875 units
   b. 2320 units
   c. 2670 units
   d. 2840 units

Clinical Laboratory Science Announces 2002 Distinguished Author Award Recipients

Recipients of the Clinical Laboratory Science (CLS) Distinguished Author Awards are chosen by CLS readers and editorial board members. Nominations are based upon originality, quality of writing, and relevance and value to the clinical laboratory science profession. The Editorial Board of CLS is pleased to announce the following recipients of the 2002 Distinguished Author Awards:

Clinical Practice Section
Cheryl Caskey MA CLS CLSp(NCA) for her article Cultural Competency in the Laboratory published in the Fall 2002 issue of CLS.

Reports and Reviews
David L McGlasson MS CLS(NCA) for his article A Comparison of INRs after Local Calibration of Thromboplastin International Sensitivity Indexes published in the Spring 2002 issue of CLS.

Research Section
Susan J Beck PhD CLS(NCA) and Kathy Doig PhD CLS(NCA) CLSp(H) for their article An Entry-Level MS Degree in Clinical Laboratory Science: Is It Time? published in the Summer 2002 issue of CLS.

Focus Section
J Lynne Williams PhD for her article Malignancy: An Evolving Definition of a Cancer Cell published in the Winter 2002 issue of CLS.
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:

American Society for Clinical Laboratory Science
P.O. Box 79154
Baltimore, MD 21279-0154

A certificate and credit will be awarded to participants who achieve a passing grade of 70% or better. Participants should allow eight weeks for notification of scores and receipt of certificates.

Focus: Hemorrhagic Abnormalities carries 3.0 hours of advanced level credit. This form can be submitted for credit for up to one year from the date of issue.

Print or type carefully.

(01) NAME ________________________________ ASCLS membership number _______________

(02) ADDRESS _________________________________________________________________________________________

(03) CITY_____________________(04) STATE/COUNTRY _______________(05) ZIP/POSTAL CODE_________________

(06) DAYTIME PHONE (______) ____________________________(07) E-MAIL:_____________________________________

(08) CREDIT CARD # ____________________________   TYPE (CIRCLE)    AE     MC     VIS       EXP. DATE __________

Check all that apply

☐ I am an ASCLS member  
☐ I am not an ASCLS member  
☐ I would like to receive ASCLS membership information  
☐ I have previously participated in Focus  
☐ I would like information on other continuing education sources

Answers

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

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

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

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

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

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

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

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

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

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

Trends and Technology: Spring 2003

MARY JANE GORE

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

NEW PRODUCTS
American Diagnostica Inc (ADI) has introduced two separate IMUCLONE-ELISA Antiphospholipid (aPL) test kits to its broad portfolio of Special Coagulation assays. Suiiting the needs of clinical laboratory scientists and life science/drug discovery researchers, the IMUCLONE test kits can be used for rapid quantitation of IgG and IgM aPL antibody levels in either human serum or plasma. ADI also now is offering Abnormal Hemostasis Reference Plasma. The plasma has been assayed for almost 30 individual coagulation parameters, including fibrinogen, PT, APTT, AT, plasminogen, Factor VIII antigen, Protein C antigen and several other intrinsic and extrinsic factors. Contact Robert Janetschek at (203) 661-0000, ext. 34.

ADI's anti-phospolipid ELISA test kits

Tecan US, a leading player in the Life Sciences supply industry, introduced Te-MO 3/3 at Lab Automation 2003. Te-MO 3/3 is a compact, multi-channel, versatile pipetting option for the Tecan Genesis platform that significantly increases the productivity of the Tecan Genesis platform without increasing the system's footprint. Te-MO 3/3 can simultaneously execute plate loading and pipetting of 96-, 384- and 1536-well plate formats which increases the throughput of the Genesis and Freedom Workstation, while the Genesis 8-channel pipetting arm is available for more complex pipetting patterns such as hit picking. The Te-MO 3/3 is ideal for a wide range of applications including assay development and medium-throughput genomics. Contact Michele Valenta at (919) 361-5200.

Tecan has introduced its Genesis FE500 Workcell, a fully automated platform designed to meet the sample processing requirements of hospitals and other clinical laboratory sites. The new modular system offers more options in selecting modules for a perfect fit to the specific range of activities performed by each laboratory, including different options for the individual processing steps, from specimen sorting and centrifugation to barcode labeling and placing tubes in sample racks made by a wide variety of manufacturers. For more product information, e-mail dimitri.katsoulis@tecan.com and visit www.tecan.com.

Tecn workcell

VWR International, a global scientific supplies distributor, has published its 2003–2004 General Catalog, a comprehensive guide to laboratory equipment, instruments, chemicals, supplies, and services. In-depth descriptions, full-color photography, specifications, and pricing are included for over 46,000 products. The catalog features more than 2,000 new products, as well as...
many items exclusive to VWR. It includes useful technical reference charts that provide information on chemical resistance, reagent concentrations, common conversion factors, and more. Contact Robin Filiggi at (610) 430-7258. For a free copy of the new VWR International 2003-2004 Catalog, visit www.vwr.com, phone (800) 932-5000, or write VWR International Inc 1310 Goshen Parkway, PO Box 2656, West Chester PA 19380-0906.

Chicago-based Carstens Inc is the leading North American provider of patient charting systems. It offers more than 2,000 products ranging from ringbinder chartholders, charting paper, identification cards, and labels, to mobile and roto caddy chartholder storage systems, and WALLaroo, wall-mounted workstations. For detailed information about the full line of Carstens’ products, visit carstens.com.

Contact Paul Bjorneberg at pbjorneberg@nrmadv.com or call (312) 422-9500, ext. 17.

TRENDS AND TECHNOLOGY

Carstens condition alert cards

The new Class II Heraeus® HERAsafe® KS safety cabinets from Kendro Laboratory Products provide optimum product and personnel protection with an ergonomically superior design. The slanted front window, the rear-cabinet-wall digital eye-level-display, and use of a remote control for parameter setting combine to make our new generation of safety cabinets unmatched in the industry. The rear-cabinet-wall eye level display allows parameter and operating functions to be conveniently monitored from a seated position. Contact Sue Mortifoglio (203) 270-2203 or e-mail mortifsa@kendro.com.

Users of the latest edition of Saf-T-Pak’s “Comprehensive Guide to Shipping Infectious Substances and Diagnostic Specimens” CD-ROM will enjoy more features than before. Acting on suggestions from laboratory technicians, shippers and others, the 2003 disc allows answer changes prior to exam scoring. In addition, testing and training is now determined by an individual’s region and requirements. Those who use the CD for recurrent training will like the weighted pretest. More details about the CD-ROM and other training materials Saf-T-Pak produces can be obtained by calling (800) 814-7484 or (780) 486-0211; for further information contact, Garry Melnyk at 800-814-7484 or e-mail at garry.melnyk@compuserve.com.

Testing blood for the presence of a substance called C-reactive protein, in conjunction with cholesterol testing, could be an important means of identifying persons at risk of developing heart disease, according to a study published in a recent issue of the New England Journal of Medicine. The study is the largest to date on the use of C-reactive protein in cardiac risk assessment, and involved 27,939 women over the mean course of eight years. The levels of CRP associated with increased risk of heart disease are very low, and can only be detected using high sensitivity assays. Dade Behring’s N high sensitivity CRP assay was one of the first such tests in the market. Medical professionals can obtain information on cardiac hsCRP by visiting the Dade Behring web site at www.dadebehring.com.

ACQUISITIONS/SALES

Forensic Analytical Specialties, Inc. of Hayward CA announced today it has completed the acquisition of Compliance Management Solutions Inc (CMS), a provider of environmental health and safety consulting services. CMS was founded in 2002 to provide cost effective solutions to help its clients proactively manage their Environmental Health and Safety (EHS) programs. CMS will add primary areas of service to the offerings of Forensic Analytical, including environmental health and safety, lead hazard management, mold investigations and mitigation planning, indoor air quality management, training and program development, and data management. Contact Kerri Sadigh at (510) 887-8828.

Palco Labs Inc, a California-based manufacturer of healthcare products and medical devices, has announced the sale of its monitoring products division to Medi+Aid, a California corporation and manufacturer of optical sensors and healthcare products. Palco Labs’ monitoring division produces pulse oximeters to monitor blood oxygen levels that hospitals, clinics, and healthcare professionals use worldwide. The transfer of the monitoring division to Medi+Aid was to happen during the first quarter of 2003. For more information, call 1-800-346-4488 or e-mail info@palcolabs.com

COMING SOON

The nation’s leading advocate for healthcare quality and safety today called for greater reporting of the deaths of patients who contract fatal infections while being treated for other illnesses or injuries. The bulletin issued by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) advises that the deaths of patients from hospital-acquired infec-
The condition for further information, please visit www.rochediagnostics.com. Contact Melissa Ziriakus, (847) 236-7038.

Hycor Biomedical and the Diagnostics Division of Bayer HealthCare recently announced the signing of an exclusive, worldwide agreement for the development of autoimmune tests. Under the terms of the agreement, Hycor will develop and manufacture a broad menu of tests for the ADVIA Centaur Immunoassay System, and Bayer Diagnostics will retain exclusive rights to market the product. The tests will be developed using a combination of Bayer’s chemiluminescence and Hycor’s ELISA technologies, thereby replacing current manual immunofluorescence methods. Visit www.bayerdiag.com for more information.

NEW APPROACHES
In recent months the Critical Incident Analysis Group at the University of Virginia has been developing a public protection concept that extends, and significantly augments, the use of quarantine as a probable response to a bioterrorist incident. The project has allowed CIAG to draw on the expertise of many of its constituents and to develop its relationships with others, including the National Security Health Policy Center, the State Capitol Police, and the American Bar Association. This work is reflected in a monograph titled: “What is to be Done? Emerging Perspectives on Public Response to Bioterrorism,” published in October 2002. Requests for this publication should be directed to ciag@virginia.edu, or write CIAG at PO. Box 800657, Charlottesville, VA 22908-0657.
The 71st Annual Meeting of the American Society for Clinical Laboratory Science
July 22-26, 2003, Philadelphia, Pennsylvania

Scientific Sessions Include:
Lab Utilization in the Era of Personnel Shortages: Practical Strategies * Meeting the Requirements of Training & Competency in Today’s Clinical Microbiology Lab * Proteomic Analysis of Malignant Lymphomas * proBNP and the Patient with Heart Failure * Hospital & Blood Center Communication * and More!

Workshops & Roundtables Include:
Update on Coding, Billing, Reimbursement & Compliance * Body Fluid Cell Counts & Morphology * Lab Ergonomics Awareness * How to Avoid the Most Common Survey Deficiencies * HIPAA -- One Year Later: The Impact on Clinical Lab Operations * and More!

Professional Society Development Track

Advanced Training Institute: Human Resource Management - advance registration required by May 12th!

Visit the ASCLS homepage for details & online registration at www.ascls.org or send an email request for information to ascls@ascls.org.

“Life, Liberty and the Pursuit of Laboratory Excellence”