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The American Society for Clinical Laboratory Science serves as the voice of all clinical laboratory professionals, creating a vision for the advancement of the clinical laboratory practice field, and advocating the value and role of the profession ensuring safe, effective, efficient, equitable, and patient centered healthcare.

AMERICAN SOCIETY FOR CLINICAL LABORATORY SCIENCE

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Times Change and So Do People

SUSAN J. LECLAIR

The winter issue of the journal is the time when there is a change in the Editorial Board. This year we say goodbye to our Editor-in-Chief, Bunny Rodak. For those of you who know her, it will not be surprising to you to know that she has been a hard working, efficient leader of the Editorial Board and has infused the journal with her perceptions of the needs of an ever changing discipline and profession. For the past three years she has guided the journal through a number of updates and challenges.

Recently she formulated a plan to assist first time authors as they dared the publication process. Besides adding writing mentors to the journal, she has made it possible for people to be assisted through the sometimes winding path to authorship. She created a stronger group of Consulting Editors who will help in not only providing assistance to authors but also to provide a sounding board for potential authors and to evaluate proposed journal updates or policies.

Thank you, Bunny.

David McGlasson is also stepping down as Research Section Editor. He agreed to stay longer than his designated term in order to provide a smooth transition for his successor, MariBeth Flaws. Since we can't let him just walk away, David will be serving as a Consulting Editor. Thanks to David, our research section has become more robust and addresses a wider range of topics.

Thank you, David.

Similarly, George Fritsma has handed his role as Focus Section Editor over to Suzanne Campbell. George was critical in the development of updated author guidelines and practices which have made the process for author more efficient and clear. As with David, he doesn't get off so easily either and will be part of the Consulting Editors group.

Thank you, George.

Elizabeth Kennimer Leibach will continue to serve as the Education Editor, a position developed during Ms. Rodak's term. The ability to have an issue devoted to the research and reports of our members in education has greatly improved the journal's ability to serve our members and readers. Perry Scanlan will continue to serve as the Clinical Practice Editor, helping to create a body of real life phenomenological case studies for us.

Thank you to both Elizabeth and Perry for continuing to serve in these vital roles.

All of these editors, in addition to the routine assignments from the journal, have found the time to present educational workshops on writing, reviewing, study design, and plagiarism at the yearly Clinical Laboratory Educators Conferences and the ASCLS Annual Meetings. Their work has helped to improve the journal and provide high quality and timely information for readers.

Susan J. Leclair
Editor-in-Chief

Scope of Practice

Background

The current healthcare environment is one of considerable uncertainty, unprecedented change and limitless potential. The advances in medical research, genetics, and molecular methodologies, provide great opportunities for scientific and clinical advances in the detection, treatment and prevention of disease. Although there is great promise, the continuing changes in healthcare delivery and financing will have a profound impact on the availability and utilization of both new and existing diagnostic services.

The dynamic scientific, economic and regulatory environment has provided expanded roles for non-physician healthcare professionals, including medical laboratory scientists. This environment will allow the important issues of cost containment, access, quality, patient safety and appropriate utilization of laboratory services to be more effectively addressed. While the cost of healthcare continues to rise, there are significantly increased expectations from clinicians and healthcare consumers to deliver information in a manner that will facilitate rapid diagnosis and treatment.

At a time when the cost of healthcare is over 17% of the Gross Domestic Product (GDP), clinical laboratory services continue to provide a significant level of value.[1] Healthcare is being impacted by evidence-based medicine (EBM), evidence-based practice (EBP), and comparative effectiveness research (CER). Laboratory information provides significant data and scientific information that contribute to the development of EBM, EBP, and CER.[2] As healthcare becomes more evidenced based, and that evidence is provided by medical laboratory professionals, it is important that medical laboratory scientists assume their role as members of the interprofessional healthcare team. It is time for the appropriately qualified healthcare professionals to provide the most efficient and effective level of care by advancing independent and collaborative roles. Ultimately, by having each specific healthcare professional play their most appropriate role the total care provided to the consumer will be optimized. Appropriate utilization of healthcare resources through interaction and contributions of the interprofessional healthcare team can contribute to avoiding unnecessary invasive procedures, decrease cost, and improve patient safety. In today's complex delivery system, maximizing the effective delivery of all components of healthcare will help address the rapidly escalating cost, patient safety, and issues of access to quality and affordable healthcare.

The Medical Laboratory Science Profession

In previous statements of opinion, policy and positions, the American Society for Clinical Laboratory Science (ASCLS) has established that medical laboratory science is a profession: distinct from the practice of medicine; characterized by its own Body of Knowledge and Scope of Practice; which certifies its own practitioners; and requires of its practitioners competency in scientific, technical, managerial and scholarly principles, and high standards of performance and professional conduct.

ASCLS defines the profession of medical laboratory science as encompassing the design, performance, evaluation, reporting, interpreting, and clinical correlation of clinical laboratory testing, and the management of all aspects of these services. Clinical laboratory tests are utilized for the purpose of diagnosis, treatment, monitoring and prevention of disease. The profession includes generalists as well as individuals qualified in a number of specialized areas of expertise including microbiology, virology, hematology, immunology, transfusion medicine, clinical chemistry, endocrinology, toxicology, cytogenetics, and molecular diagnostics. Integral features of each of the specialties include diagnostic testing, research, consultation, education, information management, marketing and administration. ASCLS has a professional code of ethics that sets forth the principles and standards by which medical laboratory professionals practice.

Description of the Scope of Practice

Medical laboratory professionals, as members of the healthcare team, contribute to the prevention of disease, and the diagnosis, treatment, and prognosis of pathophysiological conditions in humans. Medical laboratory personnel are responsible for assuring reliable and accurate laboratory test results.

Quality clinical laboratory testing is evidenced by: performing the correct test, on the right person, at the right time, producing accurate test results, with the best outcome, in the most cost-effective manner. This is accomplished by:

- Ensuring that appropriate clinical laboratory tests are ordered;
- Procuring clinical laboratory test samples in an efficient, timely manner;
- Producing accurate clinical laboratory test results;
- Correlating and interpreting clinical laboratory test data;
- Disseminating clinical laboratory test information to clinicians and patients in a timely manner;
- Evaluating the outcome of clinical laboratory testing for each individual patient and the entire health care system;
- Utilizing qualified medical laboratory personnel.

The practice of medical laboratory science requires:

- Assessing, designing, evaluating and implementing new clinical laboratory test methods;
- Evaluating the appropriateness of existing and new clinical laboratory methods for clinical utility, cost-effectiveness and cost-benefit analysis;
- Developing, implementing, and reporting results of clinical laboratory research;
- Designing and implementing cost-effective delivery models for clinical laboratories, including their services and personnel;
- Developing and implementing a comprehensive Quality Management System to include:
    - Quality control and assurance of clinical laboratory testing services;
    - Competency assessment of personnel;
    - Integration with other aspects of the healthcare delivery system for ensuring appropriate utilization of clinical laboratory testing services;
    - Continuous process improvement activities to effectively utilize human resources.
- Designing, implementing and evaluating academic curricula for the education of new medical laboratory professionals;
- Designing, implementing and evaluating academic curricula for advanced education of medical laboratory professionals;
- Designing, implementing and evaluating continued education activities and career growth opportunities for medical laboratory professionals;
- Promoting awareness and understanding of the use of the clinical laboratory.

Description of Current Practice

The following scenarios describe specific examples of the scope of practice of clinical laboratory science.

Providers of Clinical Laboratory Services

Medical laboratory scientists qualified by education and experience, perform laboratory tests and provide test results to clinicians and to consumers upon request or upon clinician referral. These services can be used to assess wellness and identify disease risk factors, as well as assisting in the diagnosis, monitoring and treatment of disease. Medical laboratory scientists exercise prudence and judgment to ensure that such services are consistent with good practice and sound professional ethics. In addition, medical laboratory scientists may own or operate laboratories.

Directors of Full-Service Clinical Laboratories

Medical laboratory scientists, with appropriate graduate education, can direct full-service clinical laboratories. This function is firmly grounded in (a) applicable state law, and (b) federal regulations governing clinical laboratories under the Clinical Laboratory Improvement Amendments of 1988 and laboratory participation in Medicare and Medicaid.

Consultants for Clinical Laboratory Services

Medical laboratory scientists may appropriately provide assistance and advice to clinicians, manufacturers, and consumers of clinical laboratory testing services about the:

- Design and service scope of clinical laboratories;
- Appropriate utilization, selection and sequencing of clinical laboratory tests;
- Clinical correlations and interpretations of the quality and utility of specific laboratory results in collaboration with clinicians;
- Design and development of clinical laboratory instruments, test kits and other components;
- Appropriate use, maintenance, quality assurance and other procedural and informational requirements.

Levels of Practice

There are hierarchical levels of practice, based upon education and experience, for each of the three areas of medical laboratory science practice: scientific, managerial and educational. Specific knowledge and experience are required for each level of practice within the three areas. An individual is eligible to practice at various levels after acquiring additional experience, education, and demonstrating competence. Specific knowledge and experience is common to all three areas; none is mutually exclusive of the other. Demands of the health care environment often require an individual to practice in more than one area, thereby performing at different levels of practice.

The *scientific function* includes the production of test data, monitoring the accuracy, precision and utility of laboratory testing, the correlation and interpretation of test data, and the design, evaluation and implementation of new laboratory test methods.

The *managerial function* includes managing all aspects, technical, fiscal, workflow, and human resources, of laboratory operations.

The *educational function* includes the establishment and management of educational programs for new and current medical laboratory practitioners, other healthcare providers and consumers.

Qualifications for Practice

ASCLS believes that personnel standards should be prescribed for **ALL** personnel including directors, supervisors, medical laboratory scientists and other laboratory technical personnel to insure the accuracy and reliability of test performance. ASCLS supports the concept of the regulatory complexity model, and believes that personnel standards at the technical levels must be defined in terms of qualifications needed to perform testing at CLIA defined complexity levels.

- The individual qualified to perform CLIA waived tests must demonstrate competency, and would perform simple tests requiring little to no independent judgment and interpretation.
- The individual qualified to perform CLIA moderately complex tests must demonstrate competency as a medical laboratory technician and would perform, under direct supervision, more technically demanding tests with some degree of independent judgment and interpretation.
- The individual qualified to perform CLIA highly complex tests must demonstrate competency as a medical laboratory scientist, and would perform more technically complex tests requiring considerable amounts of independent judgment and interpretation.

ASCLS supports utilization of validated competency-based credentialing examinations for all laboratory practitioners performing moderate and high complexity testing. Waived testing should be performed by properly trained personnel. A certified medical laboratory scientist and or technician should conduct this training.

ASCLS supports the use of benchmarks to more succinctly typify positions at various levels of work and different types of work presently performed by practitioners in the clinical laboratory. A benchmark is defined as something that serves as the standard by which others may be measured.

- The benchmark for the medical laboratory scientist is the baccalaureate degree as awarded by a regionally accredited college/university including or in addition to successful completion of a medical laboratory science program accredited by an agency recognized by the U.S. Department of Education.
- The benchmark for the medical laboratory technician is the associate degree as awarded by a regionally accredited college/university including successful completion of a medical laboratory technician program accredited by an agency recognized by the U.S. Department of Education.
- The benchmark for personnel performing CLIA waived testing is successful completion of appropriate training for testing at this level. It is within the scope of practice of a certified medical laboratory scientist and/or technician to provide this training.

ASCLS supports the concept of equivalent routes for the medical laboratory scientist only in combination with a baccalaureate degree as defined by the Board of Certification (BOC).[3]

ASCLS supports the concept of equivalent routes for

the medical laboratory technician only in combination with an associate degree as defined by the BOC.[3]

ASCLS supports the concept of equivalent routes for scientists in discipline specific areas of clinical laboratory science as defined by the BOC.[3]

Experience requirements for the medical laboratory scientist and technician equivalent routes incorporating full-time medical laboratory experience for equivalency must include **ALL** major disciplines typically required in the clinical component of a medical laboratory science education program accredited by an agency recognized by the U.S. Department of Education. If the credential is discipline specific, the clinical component must contain full-time experience in that discipline. Such experience shall be under the supervision of a certified medical laboratory scientist in a CLIA certified laboratory.

ASCLS supports the concept of career mobility (ladder) which includes utilization of validated competency-based credentialing examinations and documentation of continuing education to determine competency of personnel at all levels of responsibility.

ASCLS believes that all practitioners should demonstrate continued competence through recertification and/or documentation of continuing education as stated by the BOC.[3]

Summary

The current economic and regulatory healthcare climate benefits from the roles described for medical laboratory scientists and technician. These roles achieve high quality, cost-effective assessment, diagnosis, treatment, and prevention of disease to meet the needs of a changing healthcare environment.

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This position paper was approved by the 2012 American Society for Clinical Laboratory Science House of Delegates.

Value of Clinical Laboratory Services in Health Care

Introduction

Clinical laboratory professionals and the services they provide contribute to maximizing the effective delivery of care in today's complex healthcare system. Appropriate utilization of clinical laboratory services enhances the ability of clinicians to make evidence-based diagnostic and therapeutic decisions for their patients using the optimal level of resources and minimizing overall healthcare expenditures. Clinical laboratory services are the most cost effective, least invasive source of objective health information in disease prevention and diagnosis, improving patient outcomes, assuring patient safety, and fulfilling essential public health surveillance functions. Thus, clinical laboratory professionals and the services they provide are essential in providing care that is safe, effective, patient-centered, timely, efficient, and equitable.

Background

Within the United States, laboratories are certified by the Centers for Medicare and Medicaid Services (CMS) under the CLIA (Clinical Laboratory Improvement Amendments) program. The hundreds of thousands of certified laboratories registered under CLIA are responsible for performing billions of laboratory tests annually. As the healthcare delivery system continues to evolve and more individuals seek healthcare, the role of clinical laboratory services will become increasingly more vital. Clinical laboratory testing will continue to be a significant component of the healthcare market, due to factors such as the continued improvements in technology, the development of new tests which are more specific to certain diseases, an aging patient population, and expanded consumer access to healthcare. As a result, clinical laboratory information will continue to have a major impact on evidence-based, decision-making strategies utilized in healthcare and public health.

Rationale

Impacting diagnosis, identifying abnormalities: Laboratory professionals produce accurate, sensitive and specific information using the most current technologies to guide clinical decision-making. It is the role of laboratory professionals to inform physicians about which tests have the highest effectiveness in given clinical conditions. Through this partnership, the overall cost of quality care is optimized.

Laboratory information has profound impact on patient diagnosis and prognosis. Tests, whether classified as screening or diagnostic, are essential elements of protocols used to diagnose and manage specific diseases and conditions. Testing algorithms, designed collaboratively with clinicians, are used to guide the diagnostic process, obtain the right information at the right time for a given patient, shorten the time to diagnose, decrease the length of stay and improve utilization in health care. These algorithms assure appropriate patient outcomes by assisting in rapid identification and assessment of disease severity, and creation of a therapeutic plan.

Patient Care Management: The ability of a patient care provider to consistently make timely and evidence-based decisions that impact care management is directly related to the availability of accurate, reliable and timely laboratory testing. Examples that illustrate the laboratory's value and ability to be the primary enabler of therapeutic efficacy include:

- Monitoring the effectiveness of parenteral nutritional treatment
- Ensuring proper dosing of medication
- Monitoring harmful effects of therapeutic drugs
- Identifying the causes of infection
- Determining effective antibiotic therapy
- Monitoring the effectiveness of cancer therapy
- Providing real-time decision support

Clinical laboratory services provide the information required by a physician to start, adjust or discontinue a course of treatment. This allows for the most appropriate, cost effective use of medications and other therapies, and allows the patient to be treated at the most appropriate level of care for the most appropriate period of time. Without laboratory information, these decisions would be compromised.

Patient Safety and Impacting Patient Outcomes: Effective use of laboratory services prevents medical errors, assures patient safety and identifies threats to the public. Examples that illustrate this include:

- Bioterrorism surveillance
- Providing testing prior to the initiation of medical interventions which determines the ability of the patient to undergo those treatments
- Assessing the safety of the national blood supply and tissues for transplantation
- Testing that aids in the prevention, identification and tracking of hospital-acquired infections, sexually transmitted disease, and infectious disease epidemics.

Clinical laboratory services are a vital component of ensuring optimal outcomes for all patients accessing health care, whether for simple situations or for complicated, potentially life threatening conditions - including end-of-life care. Patient outcomes are influenced by the laboratory professional's ability to provide the needed information for clinicians to monitor the effectiveness of ongoing treatments and therapies. Laboratory services assure that the health and wellness of patients with chronic illness are managed appropriately and that complications are prevented. As an example, diabetics rely on laboratory support to monitor the long-term effectiveness of their home monitoring systems. Laboratory professionals provide vital information to assist clinicians in making decisions that affect quality of life for their patients.

Patient outcomes are improved through scientific data assessment provided through laboratory services to measure quality performance of physicians and health care provider organizations, establishing care pathways, and enabling research to introduce innovative change into medicine and public health.

Positions

1. ASCLS believes that clinical laboratory services are essential components for ensuring optimal patient health outcomes and enabling safe, effective, patient-centered, timely, efficient, and equitable care.
2. ASCLS believes that interaction between laboratory professionals and healthcare providers establishes the foundation for appropriate utilization of clinical laboratory services and results in efficient and effective clinical decision-making, allowing for improvement in health outcomes.
3. ASCLS believes that laboratory professionals are essential in the ongoing innovation of advanced testing methods, evidence-based processes, information delivery and disease prevention strategies to lead improvements in healthcare and reduce unnecessary spending.
4. ASCLS believes that laboratory professionals play an essential role in wellness and prevention by providing clinical data and educational information to healthcare providers and the public and by identifying risk factors or predisposition to disease to allow for early intervention strategies.
5. ASCLS believes that laboratory professionals must meet the high standards necessary to provide quality services and protect the public's health. To that end, ASCLS supports the continued efforts to establish, in all states, licensure (or personnel standards) of clinical laboratory professionals as a means of assuring that only appropriately educated and qualified individuals are engaged in the total testing process.
6. ASCLS believes that efforts must continue to develop relationships with other healthcare provider organizations, laboratory professional associations and governmental agencies to assure that there is an adequate laboratory workforce to meet the increasing demands of a changing healthcare environment.

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This position paper was approved by the 2012 American Society for Clinical Laboratory Science House of Delegates.

Recruitment and Retention Strategies for Hospital Laboratory Personnel in Urban and Rural Settings

DEREK R. SLAGLE

ABSTRACT

Laboratory directors and administrators play vital roles in the recruitment and retention processes of their employees. A total of 71 laboratory directors from hospitals across 51 counties in Tennessee responded to questionnaires regarding recruitment and retention strategies. Respondents reported strategies for recruitment and retention, which were agreed to be effective by management. Overall, these major strategies were consistent regardless of geographic location and limited differences were noted with regard to urban-rural locations. The findings that varied significantly between urban and rural locations included: 1) rural employees needed additional supervision; 2) rural hospitals relied on local residents more so than urban hospitals; 3) rural laboratory administrators noted more limited access to resources; and the 4) lower effectiveness of recruitment agencies and family relocation programs for rural hospitals. This is significant given the disparities often associated with rural areas, and the potential to develop more successful recruitment and retention strategies for those areas Active managers in clinical laboratory science programs in the hospital setting should note effective strategies for both, recruitment and retention of personnel, and note the potential impact of geography on such processes.

ABBREVIATIONS: NAACLS-National Accrediting Agency for Clinical Laboratory Science, MLS-medical laboratory scientist, MLT-medical laboratory technician,

INDEX TERMS: Laboratories, Hospital; Personnel Administration, Hospital; Urban Health Services; Rural Health Services

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INTRODUCTION

On national, state, and local levels, there is a need for an understanding of effective recruitment and retention strategies of laboratory personnel. Changes in technology and diagnostic practices are encouraging more testing and spurring employment. However, approximately 40 percent of current laboratory staff is expected to retire in the next ten years.[1-3] In addition, continued and significant national shortages and dramatic declines in medical technology programs are all impacting the medical laboratory profession directly and therefore reinforces the importance for laboratory administrators to understand strategies for recruitment and retention of laboratory personnel.[1-3]

Nationwide laboratories require 5,000 new laboratory technologists each year to maintain optimal staffing levels; however, schools are graduating only 1,500 technologists per year, which falls well short of the demand.[4] National program trends also mirror the fact that there are fewer professionals are entering the workforce.[6] This national decline in programs is also seen at statewide levels. From personal communication to all NAACLS accredited and approved programs it was determined that Tennessee graduated 51 Medical Technologists and 77 Medical Laboratory Technicians in 2010-2011 through five programs offering MLS and five programs that offer MLT degrees.[1,7] Nonetheless both, technologists and technicians employment, are expected to grow by 14 percent between the years of 2008 and 2018; job opportunities are expected to be excellent since anticipated job openings will exceed the number of applicants; and most job openings are still expected to occur in the hospital setting.[3]

Previous studies of clinical laboratory personnel have been conducted to examine connections between recruitment, retention, and turnover. Beck and Doig

(2005) found five reasons why employees left their jobs: 1) new laboratory job, 2) moved/family obligations, 3) retirement, 4) left the field entirely, and 5) employee was fired.[8] Over 60% of these employees left their job within the first five years. Gardner and Estry (1990) surveyed laboratory managers and found that turnover, rather than low applicant numbers, contributed to a shortage at the end of the 1980's.[9] Whereas other studies of laboratory managers revealed strategies for retention that included autonomy and employee control, higher salaries, better recognition, career advancement, reduced stress, and development of better work hours.[10,11] Previous surveys of laboratory professionals recognized salary as a major factor affecting employee satisfaction and retention; whereas poor benefits, high stress, poor advancement opportunities, and deficiencies in recognition allowed for employee attrition.[12-17] What these studies have not addressed are recruitment and retention strategies from the management perspective, nor the potential impact of geography upon these processes.

Furthermore, examination should be given specifically to rural areas since these factors impact secondary health indicators such as availability and development of services offered in communities as well as availability of employment.[18] This shortage of healthcare professionals in rural communities is a problem that poses serious complications to equitable healthcare delivery. These geographically skewed distributions of healthcare professionals are favoring more urban and wealthier areas, despite the fact that people in rural communities experience more health related problems.[19]

METHODS AND MATERIALS

A thorough literature review was used to generate the strategies for recruitment and retention of laboratory professionals included in the survey tool. Participants included laboratory directors in Tennessee hospitals who were identified using the American Hospital Association Guide 2009 and the Hospital Blue Book 2009 South edition. In order to ensure accuracy regarding proper contact information and hospital information only hospitals appearing in *both* selection tools were included. The questionnaire was mailed out in three waves over a four week time period. The mailings included the survey tool, a cover letter explaining the purpose of the study, assurance of respondent confidentiality, and a self-addressed stamped envelope for questionnaire return.[20] The returned questionnaires were organized according to an internal coding system and the data were input into SPSS (Statistical Package for Social Studies) Version 18. Frequency distributions were converted to percentages of the total responses to facilitate reporting. Descriptive statistics allow the researcher to measure perception of laboratory administrators on survey questionnaire items related to recruitment and retention strategies of their hospitals, specifically focusing on rural and urban differences.

Urban and rural areas were identified according to United States Census Bureau categories. The Census Bureau classifies as "urban" all territory, population, and housing units located within an urbanized area (UA) or an urban cluster (UC). An area deemed an UA and UC consists of boundaries to encompass densely settled territory of "core census block groups or blocks that have a population density of at least 1,000 people per square mile and surrounding census blocks that have an overall density of at least 500 people per square mile. In addition under certain conditions, less densely settled territory may be part of each UA or UC." Whereas, classification of "rural" consists of all territory, population, and housing units located outside of UAs and UCs.[21-23]

RESULTS

A total of 102 laboratory directors from Tennessee hospitals met the inclusion criteria and therefore received the mailed survey tools. A total of 71 laboratory directors responded to the questionnaires. Respondents were representative of 51 counties across the state, and responses represented a 69.6% response rate.

All laboratory administrators were asked to respond to a survey tool of recruitment and retention strategies, as collected through the literature review process, of what were considered effective recruitment and retention strategies. While many strategies were not considered beneficial there were, strategies selected for recruitment (Table 1) and strategies selected for retention (Table 2) agreed upon by the majority of respondents. Additionally, the administrators were asked for general information on the status of their programs, turnover, recruitment, retention, and administrative practices. From this administrative section portion there was no

strong consensus that there existed a retention problem; however, a significant percentage of all laboratory directors stated that there was a need for laboratory personnel (85.7%) and that it was difficult to recruit new health professionals (81.5%). It was also noted that there were current vacancies (69.6%) for laboratory positions and 82.8% of respondents stated there was a shortage of available employees. When prompted as to what educational levels are hired as allied health employees at hospitals, laboratory directors stated that positions are mostly filled by Bachelors degree (83%), followed by Associates degree (76%), Masters Degree (39%), Certificate (37%), Diploma (26%), and Doctoral Degree (11%).

Table 1. Laboratory Administrator Positive Responses for Recruitment Strategies

Strategy	Responses (%)	Urban Responses (%)	Rural Responses (%)
Need to increase public awareness of allied health	96.6	97.5	94.7
Accessible community college	79.6	82.9	72.2
Recruitment by word-of-mouth	73.3	75.0	69.6
Competitive benefits	72.0	73.3	69.6
Effective co-op programs with universities	64.8	63.9	66.7
Targeting technical and community colleges	63.2	59.0	72.3
Competitive wage packets	62.9	65.9	56.5
Recruitment bonuses	62.8	68.6	50.1
Online and website marketing	60.6	62.2	57.1
The hospital emphasizes diversity when recruiting	58.4	51.2	73.7
Employees are typically from a rural area	53.4	38.5	84.3
Newspapers are effective for recruitment	44.9	44.7	45.5
Family relocation programs	42.8	56.3	17.6
Professional journal advertisements	37.0	34.2	43.8
Recruitment agencies	23.9	29.1	13.3
Our institution targets non-traditional students	8.9	10.5	5.6

Table 2. Laboratory Administrator Positive Responses for Retention Strategies

Strategy	Responses (%)	Urban Responses (%)	Rural Responses (%)
Relevance of job fit	93.2	95.0	89.5
Job security	90.0	95.1	78.9
Interpersonal relationships	90.0	90.3	89.5
Job design	89.9	90.0	89.4
Clear job descriptions	88.1	90.0	84.2
Adequate orientation	83.3	82.9	84.3
Perceived self-value of employees	81.1	84.6	73.7
Sufficient on the job training	79.7	82.6	73.9
Employee decision making power	78.4	78.1	79.0
Departmental decision making power	77.5	79.5	73.7
Undergoing stressful working conditions	73.3	68.3	84.2
Individual employee's lifestyle aids in organizational retention	70.7	71.8	68.5
Implementing new technologies	60.0	65.8	47.3
Employees take advantage of professional development	56.0	61.4	45.5
Career ladder and career structure	55.2	60.0	44.5
Sufficient autonomy	54.2	52.5	57.9
Resources are unevenly distributed or limited	53.3	46.3	68.5
Employees experience burnout	45.2	41.5	52.7
Our employees need regular supervision	45.0	36.5	63.2
Management issues causes turnover	37.3	32.5	47.4
There are adequate opportunities for professional development	36.5	50.0	39.1
Work intensity is too high	35.6	35.0	36.9
Policy issues increase turnover	23.4	21.9	26.3
Chronicity and severity of clients' illnesses lead to turnover	15.9	12.8	22.7

DISCUSSION

There were strategies for recruitment where over half of all respondents agreed on their effectiveness regardless of geographic location: (1) there is a need to increase public awareness of allied health; (2) accessible community college; (3) recruitment by word-of-mouth; (4) competitive benefits; (5) effective co-op programs with universities; (6) targeting technical and community colleges; (7) competitive wage packets; (8) recruitment bonuses; (9) online and website marketing; and (10) the hospital emphasizes diversity when recruiting. Similarly, strategies were noted for retention where over half of all respondents agreed on their effectiveness when regardless of geographic location: (1) relevance of job fit; (2) job security; (3) interpersonal relationships; (4) job design; (5) clear job descriptions; (6) adequate orientation; (7) perceived self-value of employees; (8) sufficient on the job training; (9) employee decision-making power; (10) departmental decision-making power; (11) undergoing stressful working conditions; (12) individual employee's lifestyle aiding in organizational retention; (13) sufficient autonomy. Overall, these major strategies were consistent regardless

of geographic location and limited differences were noted with regard to urban-rural locations. However, laboratory management did cite key differences on select strategies in favor of geographic locale.

There is strong evidence from various studies that 'rural origin' (or rural background) is associated with rural practice.[19,24-28] In this study, rural responses (84.3%) doubled that of urban responses (38.5%) when asked if they believed employees are typically from a rural area. This indicated that laboratory personnel working in a rural setting are more likely from a rural area than their counterparts, which leads to the implication that rural laboratory directors should potentially focus recruitment efforts locally or at other rural applicants when filling open positions. Initiatives aimed at local technical and community colleges or programs associated with nearby universities may be beneficial methods for rural recruitment. Further, concentrated efforts aimed at targeting residents of rural locations such as word-of-mouth and efforts of locally targeting members of the community may be beneficial in attracting applicants to positions in rural hospitals. Also, there were some differences in perceptions from rural (68.5%) and urban (46.3%) respondents when questioned about limited or unevenly distributed resources at their hospitals. Within hospitals there are multiple demands for access to capital, the maintenance of the facilities, and for advancements in technologies. These rural respondents are reporting that the funding for laboratory services is limited and there can be waiting lists that impact the services provided. For these laboratory professionals there can be a lack of opportunities to practice and develop job-related skills, frustrations over funding and facilities, and decreased services to patients; all of which can impact retention of the employees. This fact should not be surprising given the perception of disparities of rural hospitals compared to urban locations or the actual, greater access to management resources associated with some urban localities. Urban managers could potentially highlight these resource advantages for urban areas when applicants are from more suburban or rural areas. Similarly, a large number of comments from hospital administrators noted the difficulty of suburban hospitals when competing with accessible urban hospitals.

Family relocation programs for new recruits was an area where there was a discrepancy in responses for urban (56.3%) and rural (17.6%) responses. Similarly, if looking at the negative responses from laboratory directors, rural administrators stated, (53.4%) recruitment agencies were ineffective when just 22.6% of urban respondents disagreed with the statement. Further, examination into the impact of urban and rural on recruitment initiatives could increase both the efficiency and effectiveness of the hiring process for laboratory personnel, especially if resources are limited.

Rural laboratory respondents reported (63.2%) at a much higher rate than their urban counterparts (36.5%) when asked if their employees needed additional supervision. Examination of management practices, employee autonomy, and adequate orientation, training, and education should be conducted in order to address the issue that managers stated their employees needed additional supervision based upon geographical location.

CONCLUSION

This study highlighted strategies for recruitment and for retention of laboratory personnel as reported by administrators across Tennessee hospitals. Current managers in clinical laboratory science programs in hospitals should note these strategies and potentially utilize these within their programs since the top responses for strategies weren't necessarily impacted by geography. However, certain responses saw a geographically skewed distribution of responses regarding what were effective recruitment and retention approaches for laboratory personnel. Further consideration of the potential impact of geography on the methods of laboratory management could potentially yield local remedies to shortages, changes caused by technology and diagnostic practices, and expected retirement trends. Continued examination into these findings is needed for clinical laboratory science in hospital settings regarding future trends of employment for the profession.

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The Pathology of Alcohol Use and Abuse

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ABSTRACT

Alcohol is the most widely abused substance in the United States and its pathology is responsible for more pathological conditions than all other forms of drug use combined.[1] Alcohol dependence is associated with a number of adverse individual and societal consequences[2,3] and high rates of morbidity and mortality.[4] Alcohol use and abuse have a significant pathological effect on the brain, fetus, liver, heart, pancreas, and immune system.[2] Cancer risks have also been attributed to alcohol use and abuse.[5] Assessing acute and chronic alcohol consumption is critical to effective treatment but unfortunately currently available clinical laboratory testing procedures lack the ability to inform alcohol treatment providers about use and abuse.[6]

ABBREVIATIONS: DSM-IV-Diagnostic and Statistical Manual of Mental Disorders, FAS-fetal alcohol syndrome, HDL-high density lipoprotein

INDEX TERMS: Abuse, Addiction, Alcohol Dependence, Pathology, Use.

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INTRODUCTION

Many cultures around the world use alcohol to celebrate special occasions, to socialize, and to self-medicate during trying times.[7] While alcohol is legal in the United States, the abuse of alcohol has health, social and legal implications. Alcohol abuse can have serious health effects including weakening of the immune system, contributing to cancers, and damage to internal organs.[2] Alcohol is known to affect people in different ways due to genetics, an individual's environment and diet which all culminate in the effect of alcohol on an individual and in alcohol related diseases.[4]

Alcohol consumption is measured by what is referred to as a "standard drink" and this measurement helps assess the risks an individual undertakes per given time period.[7] One standard drink contains 0.6 fluid ounces or 14 grams of pure alcohol (ethanol). Another way of viewing this is as follows:

- 12 fluid ounces of beer (about 5% alcohol)
- 8 to 9 fluid ounces of malt liquor (about 7% alcohol)
- 5 fluid ounces of table wine (about 12% alcohol)
- 1.5 fluid ounces of hard liquor (about 40% alcohol).

Using this definition, research has shown that "low-risk" drinking levels for males are four or less drinks on any single day and no more than 14 drinks per week. For females, it is three or less drinks in any single day and no more than 7 drinks per week.[7] Individuals over the age of 65 should consume no more than 3 drinks per day or 7 drinks per week.[8] It is recommended that people who should abstain from alcohol completely include those who:

- Plan to drive a vehicle or operate machinery;
- Are pregnant or trying to become pregnant;
- Take medications that interact with alcohol; and
- Have a medical condition aggravated by alcohol.[8]

Alcohol use is categorized into three categories.[10] These are use, abuse, and addiction (DSM-IV uses the word dependence instead of addiction). The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) is a coding manual published by the American Psychiatric Association that includes all currently recognized mental health disorders. Use is defined as the consumption of alcohol in a social manner that does not create problems for the user. Abuse is the misuse of alcohol that leads to intoxication and/or behavior that is counterproductive for the individual. Addiction on the

other hand is a state where the individual becomes dependent on alcohol and feels a need to consume alcohol which often leads to tissue destruction.

Alcohol Effects on Health

The brain is an elaborate network of connections that drive the physical and psychological processes. Alcohol causes a disruption that can have long lasting consequences by changing the way the brain works which results in an array of assorted problems.[11] The structure of the brain is complex and it includes multiple systems that interact to support the body's functions including cognition, breathing, and muscle movement.[12] The multiple brain structures communicate with each other through over a trillion nerve cells termed neurons.[12] Neurons in the brain transform information into electrical and chemical signals the brain can understand.[12]

Chemicals termed neurotransmitters carry messages between neurons.[12] The brain is constantly balancing the neurotransmitters that speed things up and the ones that slow things down to create stability.[12] Alcohol can alter the tempo of communication between neurotransmitters in the brain.[4] Heavy alcohol consumption (even on a single occasion) can disrupt the delicate balance of neurotransmitters.[11] Acute alcohol intake can cause sluggish neurotransmitter relays causing extreme drowsiness, trigger mood and behavioral changes, including depression, agitation, memory loss, seizures and loss of executive control.[11]

There still is much that is not understood about the brain's working mechanisms and how alcohol affects those mechanisms. Researchers are constantly developing a better understanding about how alcohol interrupts communication pathways in the brain and changes the brain's structure, as well as the resulting effects on behavior and functioning.[12] Using brain imaging and psychological tests, researchers have identified the regions of the brain most vulnerable to alcohol's effects.[13] These include:

- **CEREBELLUM** – This portion of the brain controls motor coordination. Damage to the cerebellum results in a loss of balance and may affect cognitive functions such as memory and emotional response.
- **LIMBIC SYSTEM** – This system is a complex one that orchestrates a variety of tasks such as memory and emotion. Damage to the limbic system impairs the orchestration of these functions.
- **CEREBRAL CORTEX** – This region of the brain directs our abilities to think, plan, behave intelligently, and interact socially. This area also connects the brain to the rest of the nervous system. Damage to the cerebral cortex may impair memory, the ability to solve problems, and to learn.

Long-term, heavy drinking causes changes in the actual neurons including reductions in the size of brain cells.[11] As a result brain mass shrinks and the brain's inner cavity grows bigger.[12] These changes often affect a wide range of abilities, such as motor coordination; temperature regulation; sleep; mood; and various cognitive functions, including learning and memory. One neurotransmitter particularly susceptible to even small amounts of alcohol is the glutamate which affects memory.[14] Researchers believe that alcohol interferes with glutamate action, resulting in temporary "black outs," or forgetting much of what happened during a night of heavy drinking.[14] This mechanism also accounts for gradual loss of other cognitive functions.

The brain tries to compensate for these disruptions by having the neurotransmitters adapt to the presence of alcohol.[14] Unfortunately, these adaptations can have negative results, including alcohol tolerance, developing alcohol dependence, and experiencing alcohol withdrawal symptoms.[14]

Liver Damage that Affects the Brain

Liver disease due to alcohol abuse also damages the brain.[15] This is because the liver breaks down alcohol and its associated toxins. During this process, alcohol's byproducts damage liver cells so they no longer function as well as they should they then allow toxic substances (ammonia and manganese in particular) to travel to the brain.[15] These substances cause a serious and potentially fatal brain disorder known as hepatic encephalopathy.[16] Hepatic encephalopathy causes a range of problems including:[16]

- Sleep disturbances;
- Mood and personality changes;
- Anxiety;
- Depression;
- Shortened attention span;

- Coordination problems, including asterixis, which results in hand shaking or flapping;
- Coma; and
- Death.

Hepatic encephalopathy can be treated with compounds that lower blood ammonia concentrations and medical devices that remove harmful toxins from the blood.[16] In severe cases, patients suffering from hepatic encephalopathy require a liver transplant.[16]

Fetal Alcohol Spectrum Disorders

Alcohol can affect the brain at any stage of development including during fetal growth. Fetal alcohol spectrum disorders include the full range of physical, learning, and behavioral problems, and other birth defects.[17] The most serious of these disorders is the fetal alcohol syndrome (FAS), which is characterized by abnormal facial features and is associated with severe reductions in brain function and overall growth.[17] The Centers for Disease Control report that FAS is the leading preventable birth defect associated with mental and behavioral impairment in the United States today.[18]

The brains of children born with FAS are smaller than normal and contain fewer cells and neurons.[19] Current research is studying whether the brain function of children and adults with FAS can be enhanced with complex rehabilitative training, dietary supplements, or medications.

Effects on the Heart

Alcoholic Cardiomyopathy - Long-term heavy drinking will weaken the heart muscle, resulting in a condition called alcoholic cardiomyopathy.[20] A weakened heart droops and stretches and it is therefore unable to contract effectively.[20] As a result, the heart cannot pump enough blood to sufficiently nourish the organs causing severe damage to organs and tissues. Symptoms of cardiomyopathy include shortness of breath and other respiratory issues, fatigue, swollen legs and feet, and an irregular heartbeat that can lead to absolute heart failure.[20]

Arrhythmias – Even binge drinking can affect the pace at which a heart beats.[21] The heart's internal pacemaker keeps the heart pumping consistently and at the appropriate speed. Often alcohol disrupts the pacemaker causing the heart to beat rapidly or irregularly.[21] These heart rate abnormalities are referred to as arrhythmias. The two types of alcohol related arrhythmias are:[21]

- **ATRIAL FIBRILLATION** – During this arrhythmia, the heart's upper, or atrial, chambers shudder weakly but do not contract as they should. Blood can pool and/or clot in the upper chambers. If a blood clot does develop and travels to the brain, a stroke occurs. Should the clot travel to other organs such as the lungs, an embolism occurs.

- **VENTRICULAR TACHYCARDIA** –This arrhythmia occurs in the heart's lower, or ventricular, chambers. Electrical signals travel through the heart's muscles, prompting contractions that keep blood flowing. Alcohol-induced damage to heart muscle may cause electrical impulses to loop through the ventricle too many times, causing excessive contractions. As a result the chambers do not fill up with sufficient blood between each beat. In turn the rest of the body does not get enough blood causing dizziness, lightheadedness, unconsciousness, cardiac arrest or sudden death.

Strokes

When blood cannot reach the brain a stroke occurs. About 80 percent of strokes are the result of a blood clot preventing blood flow to the brain.[22] These are termed ischemic strokes. Another reason for a stroke is blood accumulating in the brain or in the spaces surrounding the brain and these are termed hemorrhagic strokes.[22]

Clinicians attribute binge drinking or long-term heavy drinking to strokes regardless of a history of coronary heart disease.[23] Recent studies show that those who binge drink are 56% more likely than persons who never binge drink to experience an ischemic stroke.[23] Binge drinkers also are about 39% percent more likely to suffer any type of stroke when compared to people who never binge drink.[23] It should be noted that alcohol exacerbates the problems associated with strokes such as hypertension, arrhythmias, and cardiomyopathy.[23]

Hypertension

Chronic alcohol use, as well as binge drinking, can lead to hypertension.[23] A healthy blood vessel is elastic in nature, stretching as the heart pumps blood through the vessel. Hypertension occurs when the blood vessels stiffen, making them less flexible, thus increasing the pressure within the vessels. Adding to this event heavy alcohol consumption prompts the release of certain stress hormones that constrict blood vessels.[23]

Research shows that healthy people who drink moderate amounts of alcohol may have a lower risk of developing coronary heart disease than nondrinkers.[5] This research defines moderate drinking as no more than two drinks in a given day for men and one drink per day for women who are not pregnant or trying to conceive.

Other recent studies show that moderate drinking helps inhibit and reduce the build-up of fat in the arteries.[24] It can also raise the levels of HDL in the blood, which help prevent heart disease. Moderate drinking can help safeguard against heart attack and stroke by averting blood clots from forming and by dissolving blood clots that do develop.[24] Drinking moderately has also been shown to help keep blood pressure levels in check.[24]

Careful consideration must be given to these benefits because they may not apply to people with existing medical conditions, or who take certain medications. An important note is that researchers discourage people from initiating moderate drinking simply for the health benefits.[24]

Biliary Effects

Morbidity and mortality statistics show that liver disease is one of the leading causes of illness and death in the United States.[25] Alcohol abuse is attributed to more than 2 million Americans suffering from liver disease.[25] For the most part liver disease strikes people who abuse alcohol over many years.

Risk factors tend to be individualistic and include gender, genetics, alcohol accessibility, social customs pertaining to drinking, cigarette smoking, obesity and poor diet.[26,27] These can affect a person's susceptibility to alcoholic liver disease. Research shows that about one in five heavy drinkers will develop alcoholic hepatitis, while one in four will develop cirrhosis.[27]

One of the foremost functions of the liver is to store energy and nutrients.[26] The liver also produces proteins and enzymes that thwart disease.[26] Perhaps the best known function of the liver is to rid the body of dangerous substances such as toxins. The liver recognizes alcohol as a harmful substance and breaks most of it down.[26] Unfortunately, the process of breaking down alcohol generates toxins yet more harmful than alcohol itself.[26] The resulting toxins damage liver cells, promote inflammation, and weaken the body's natural resilience.[27] Ultimately, these problems will disrupt the body's metabolism and impair the utility of other organs.[27] Because the liver plays such a vital role in alcohol detoxification, it is particularly vulnerable to impairment from excessive alcohol.

One cause for fat build up in the liver is heavy drinking (even if for just a few days).[28] The result of fat building up in the liver is referred to as steatosis, or more commonly known as a fatty liver. This is the earliest stage of alcoholic liver disease.[28] The excessive fat is grueling for liver functioning and heartens the development of dangerous inflammations, such as alcoholic hepatitis.[28] Interestingly alcoholic hepatitis does not present obvious symptoms for some individuals. However, for others alcoholic hepatitis presents symptoms of fever, nausea, appetite loss, abdominal pain, and mental confusion.[28] As the severity of alcoholic hepatitis increases, the liver becomes dangerously enlarged, resulting in jaundice, excessive bleeding, and clotting complications.[28]

Fibrosis is a liver condition where scar tissue builds up in the liver as a result of heavy drinking.[26] The chemicals in the liver that are needed to break down and remove this scar tissue are altered by alcohol resulting in diminished liver function.[26] Continued consumption of alcohol promotes excessive scar tissue build up resulting in a condition called cirrhosis, which can be thought of as a slow deterioration of the liver. Complications such as jaundice, insulin resistance and type 2 diabetes, and liver cancer, may result as cirrhosis diminishes liver function.[26]

A variety of lifestyle changes can aid in addressing alcoholic liver disease. The first and most effective change is abstinence from all alcohol.[28] Abstinence from alcohol will prevent further injury to the liver. However, when cirrhosis becomes severe, a liver transplant may be the principal treatment option.[26,27]

Pancreatic Considerations

Many people who suffer from problems of the pancreas are also heavy drinkers. Pancreatitis is often caused by habitual and excessive drinking which damages the pancreas.[29] The risk of developing pancreatitis increases as excessive drinking continues over time, but only about 5% of people with alcohol dependence will develop pancreatitis.[31] For unknown reasons some people are more susceptible to the disease than others, but researchers have not yet identified what factors (e.g. genetic, environmental) contribute to the disparity.[31]

The pancreas is vital in food digestion and its conversion into fuel for the body. The pancreas delivers enzymes into the small intestine that digest carbohydrates, proteins, and fat.[30] The pancreas also secretes insulin and glucagon, two hormones that regulate the process of utilizing glucose and controlling glucose levels.[30] Alcohol consumption damages pancreatic cells and impacts the metabolic processes involving insulin.[29,30] This often leads to pancreatic inflammations.

A healthy pancreas secretes enzymes to the small intestine for the metabolizing of food. Alcohol interferes with this process by causing the pancreas to secrete its enzymes internally, rather than sending them to the small intestine.[29] These enzymes (as well as acetaldehyde*)* are harmful to the pancreas.[29] The process can cause inflammation, as well as swelling of pancreatic tissues and blood vessels. This inflammation is called pancreatitis, and it prevents the pancreas from functioning properly. Pancreatitis occurs as a sudden attack, and is referred to as acute pancreatitis.[29] If excessive drinking continues, the inflammation can become constant and worsen.[30] When this happens the condition is known as chronic pancreatitis.

Pancreatitis is also a known risk factor for pancreatic cancer.[31] Someone who is a heavy drinker may not detect pancreatic damage until they experience an attack. An acute pancreatic attack may consist of the following symptoms:

- Abdominal pain, which may radiate up the back;
- Diarrhea;
- Fever;
- Nausea and vomiting;
- Rapid heart rate; and
- Sweating.[31]

Chronic pancreatitis may trigger these same symptoms as well as blood sugar problems.[30] Chronic pancreatitis will slowly destroy the pancreas leading to diabetes or possibly death.[30]

Abstinence from alcohol can slow the progression of pancreatitis and at the same time reduce its painful symptoms.[29] A low-fat diet often helps and it is also imperative to guard against infections.[31] Treatment options are limited but include enzyme-replacement therapy and insulin, so as to improve pancreatic function.[30] Surgery is necessary to relieve pain, clear blockages, and reduce attacks in some patients.[30,31] In short, the effects of alcoholic pancreatitis can be managed, but rarely reversed.

Cancer risks

Cancer risks can be attributable to genetics, the environment, and lifestyle behaviors.[32] Effecting change to our genes and our environment is limited however, lifestyle behaviors offer an opportunity for change.

Alcohol abuse is one lifestyle behavior that can increase the risk of developing certain cancers.[32] While the risk is increased it does not mean that everyone abusing alcohol will develop cancer. Numerous studies do show the more a person drinks, the greater the risk of developing certain types of cancer.[32] For example, Italian research scientists conducted a meta-analysis on 200 studies reporting alcohol's impact on cancer risk.[33] The results show that as alcohol consumption increases, the risk for developing a variety of cancers also increases. The National Cancer Institute has identified alcohol as a risk factor for the following types of cancer:

- Breast;
- Esophagus;
- Larynx;
- Liver;
- Mouth; and
- Pharynx.[34]

Epidemiology reports show that 7 out of 10 people diagnosed with mouth cancer are heavy drinkers. Individuals who consume five or more drinks per day have risks that are associated with colon and rectal

cancers.[34] In fact, summary from the World Cancer Research Fund report indicate that women who drink five standard alcohol drinks per day have about 1.2 times the risk of developing colon or rectal cancer when compared to women who do not drink at all.[35]

Research has found that people who drink are also more likely to smoke, and the combined effect increases the risk of cancer significantly.[36] It is well documented that smoking alone is a risk factor for some cancers, but when combined with alcohol the risk intensifies the cancer-causing properties of each substance.[33]

Because alcohol and tobacco both come in direct contact with the mouth and throat, the risk of oral cancer is greater. People who drink and smoke are 15 times more prone to develop cancers of the mouth and throat than non-drinkers and non-smokers.[33,34,36] Research studies estimate that when alcohol and tobacco are used together they are responsible for:

- 80% of throat and mouth cancers in men;
- 65% of throat and mouth cancers in women;
- 80% of esophageal squamous cell carcinoma (a type of esophagus cancer); and
- 25 to 30% of all liver cancers.[34]

Alcohol effects on the immune system

The immune system is designed to protect the body from foreign substances that cause illness. The immune system is often conceptualized as a military unit because it defends the body from infection and disease. The skin as well as the mucous that lines the respiratory and gastrointestinal tracts help prevents foreign substances from entering or staying in the body. Should foreign substances somehow make it through these barriers, the immune system triggers two defensive systems; the innate and adaptive systems.[37]

The innate system exists in the body prior to any exposure to foreign substances such as bacteria, viruses, fungi, or parasites (referred to as antigens). The components of the innate system include:

- **WHITE BLOOD CELLS** – White blood cells are the first line of defense against infection. They surround and swallow foreign bodies quickly.
- **NATURAL KILLER (NK) CELLS** – Natural Killers are a specialized white blood cell that detect and destroy cells infected with cancer or viruses.
- **CYTOKINES** – White blood cells produce and direct these chemical messengers directly to an infected site. Cytokines then trigger an inflammatory response, such as dilating blood vessels and increasing blood flow to the affected area. These chemical messengers also attract more white blood cells to an infected area.[37]

The adaptive system is engaged after the initial exposure to an infection. In subsequent infections, the adaptive system is employed to attack the antigen faster and more efficiently than occurred during the first exposure.[37] The components of the adaptive system include:

- **T-LYMPHOCYTE CELLS** – T-cells reinforce the work of white blood cells by targeting individual foreign substances. The strength of T-cells is that they can identify and destroy a vast array of bacteria and viruses. They can also kill infected cells and secrete cytokines.
- **B-LYMPHOCYTE CELLS** – B-cells produce antibodies that fight off harmful substances by sticking to them and making them jut out to other immune cells.
- **ANTIBODIES** – After B-cells encounter antigens, they produce antibodies. These proteins target specific antigens and then remember how to combat the antigen.[37]

Drinking too much alcohol weakens the immune system by suppressing both the innate and the adaptive immune systems. Chronic alcohol use reduces the ability of white blood cells to effectively engulf and destroy harmful bacteria.[38] Excessive drinking also disrupts cytokines production, resulting in either excessive or insufficient amounts of chemical messengers.[38] An abundance of cytokines often cause damage to tissues, whereas a lack of cytokines increases the opportunity for infection.[38] Chronic alcohol use also suppresses the development of T-cells and may impair the ability of NK cells to assault tumor cells.[38] This reduced function makes the body more vulnerable to bacteria and viruses, and less capable of destroying cancerous cells.[38]

With a compromised immune system, chronic alcohol drinkers are more prone to contract diseases (e.g. pneumonia, tuberculosis) than people who do not drink.[39] There is also research data showing an association between alcohol's damage to the immune system with an increased susceptibility to contracting HIV.[39] It appears HIV progresses faster in chronic drinkers when compared to non-drinkers.[39] Drinking excessively on a single occasion (such as binge drinking) can also compromise the immune system.[37] Drinking to intoxication slows the body's ability to produce cytokines which produce inflammations that ward off infections.[37] Without these inflammatory responses, the body's ability to defend itself against bacteria is significantly reduced. A recent study demonstrates that slower inflammatory cytokine production can reduce the body's ability to fight off infections for up to 24 hours after intoxication.[39]

It is not known if abstinence, reduced drinking, or other measures will reverse the effects of alcohol (either partially or completely) on the immune system. Nevertheless, it is notable to remember that avoiding drinking alcohol minimizes the burden on the immune system, especially when fighting a viral or bacterial infection.

DISCUSSION

Alcohol use has a two-edge sword. It contributes to social functions and used in moderation can have health benefits. On the other hand alcohol can be destructive to the physiological systems within the human body and affecting a person's quality of life. Alcohol can cause measurable pathology in one system of the body or it can simultaneously affect multiple systems. Some of these effects, such as cancer, can be life threatening. Because alcohol is a legal drug, communicating its dangers and effecting behavioral changes has been a challenge to clinicians and public health officials.

Organizations such as the National Institute of Alcohol Abuse and Alcoholism, Mothers Against Drunk Drivers, Alcoholics Anonymous (AA), and law enforcement agencies have all undertaken efforts to educate the public to the dangers of alcohol use and abuse however the problem persists. Binge drinking on college campuses is a common problem as is drinking and driving which cause untold carnage on highways.

Behavioral treatment programs have been developed in most locations and aim to treat individuals who abuse alcohol or have become dependent. Because alcohol dependence is a chronic disease success rates vary among programs. One of the challenges the treatment programs face is the measurement of alcohol use. Most currently available clinical laboratory tests for alcohol do not offer the sensitivity and specificity required by treatment providers, so they must rely on patient self-reports which often are not accurate or truthful. More research into the development of alcohol testing (both acute and chronic) by clinical laboratory methods is needed to advance the treatment of patients with alcohol problems so as to minimize its pathology.

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Development and Feasibility of an Electronic White Blood Cell Identification Trainer

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ABSTRACT

A prototype computer-based training tool to improve WBC identification skills was developed. Students were assigned to complete five simulated WBC differentials but were allowed ample free time to use the tool at will to complete additional cases and to use the software in two alternative learning modes. The assignment was made at the end of the traditional WBC differential training activities in the first semester of hematology in the clinical laboratory science curriculum. The tool recorded usage data during the one month that students had access.

Student performance was compared to the consensus results from an expert panel of hematology instructors. Usage tracking data was extracted and reviewed. The performance data indicated that students varied in WBC identification skill on the assignment. The usage tracking data showed that students used the tool only slightly more than the assigned cases and did not use alternative learning modes.

Data from the expert panel indicated that the experts varied greatly in the number of discrepancies from the consensus opinion. Item analysis indicated the cell types that were most problematic.

The prototype experience prompted the creation of a revised subsequent version of the trainer that is now being evaluated in our CLS program. The new trainer is web-based offering personal computer and mobile device access.

ABBREVIATIONS: WBC-white blood cell; CLS-Clinical Laboratory Sciences; MLT-Medical Laboratory Technician; URL-universal resource locator (web address)

INDEX TERMS: Competency assessment; Student assessment; Psychomotor training; on-line training

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INTRODUCTION

Diligently assessing practitioner's skills in WBC identification exposes practice gaps that go undetected with less rigorous competency assessment activities.[1,2] Many labs use a selection of cell images, such as those from proficiency testing activities, to quiz employees as a method of competency assessment. Problems identified with this technique include the low number of cell examples used and possibility of "discussing" the correct answers by participants.[2] Others have cited validity questions for these methods including content validity and predictive validity.[1]

The use of electronic tools for competency assessment has been explored and appears promising.[2,3,4] Horiuchi et al used a commercially-produced competency software coupled with images from digital computer-aided microscope system to assess competency and demonstrate improvement in poor performers.[2] Burthem et al successfully piloted a digital morphology competency assessment method and delivered the package via the internet. Participant opinions of the

method were generally favorable and participants indicated that they saw potential for applications of the technique as a teaching aid.[4]

Studies show that expertise leading to expert performance is gained by deliberate practice, which in a nutshell means practice in a manner of increasing challenge as skills are gained.[5] Practicing at the same difficulty level over a long period of time will not lead to expert performance. Ericsson states that "living in cave does not make one a geologist", meaning that low-level practice and performance does not increase skill. He describes numerous studies showing performance gaps in medical skills and proposes that expertise in medicine could be improved by accessing a library of difficult challenges where one would perform diagnostic tasks and receive feedback from validated expert practitioners.[5]

Tutoring is shown to improve student performance greatly over traditional classroom instruction. Bloom calls this the "2 sigma problem" because the demonstrated increase in performance is two standard deviations. It is a problem because tutoring is impractical for all students given the restraints of time and resources. He outlines the problem of finding teaching methods that would be equivalent to tutoring and suggests that computer-based training might be a solution.[6] Studies have since confirmed this suggestion as a viable method of improving performance.[7]

In an ideal world, a student would learn WBC identification by a prolonged period of study with an expert but it is more likely that a student first learns identification with an instructor using projected images and talking through the identification process. This is typically followed by self-study on practice cases with some numerical data to compare one's performance, a limited number of assessments using study sets and ad-hoc consultation with the instructor on troublesome cells. In this way, the student becomes calibrated to the teacher and must meet a minimum level of proficiency, a variably defined grade of "C", before advancing.

In clinical training, students may encounter other faculty that may refine or recalibrate the student with such tools as double-headed microscope sessions and case studies that are assigned and discussed. Once in the workforce, the novice relies on peers, supervisors and doctoral staff for consultation and feedback. These are variable sources of unknown quality.

One limiting factor in these modes of learning is the variable skill level of the primary instructor, clinical instructors and peer consultants. The student or novice can never hope to progress beyond the skill level of the teacher.[5] Validated expert feedback is a rare commodity in many settings, as in too many labs on too many shifts, technologists have limited face-to-face time with an expert instructor, pathologist or hematopathologist.

Given this background, an electronic tool that challenges users, allows for increasing difficulty as skills are gained and provides a standard for calibrating identification skills was envisioned and proposed. It would serve as a teaching and professional development tool to address the scarcity and variable nature of expert feedback in training and in the workforce. This study sought to explore the feasibility of developing such a tool.

MATERIAL AND METHODS

Challenge Media

Cell image sequences were captured using an analog charge-coupled device camera mounted to a light microscope. The camera's composite output was digitized using an analog to digital video converter (Grass Valley ADVC-55) and the digital output recorded on a personal computer using video recording software (QuickTime Player X. Apple Corporation, Cupertino, CA). A computer script was developed to collect video for exactly 7 seconds at 30 frames per second. Once recording started, the microscope was slowly focused up and down as one would when examining a cell. The resulting movie represented an image sequence (i.e. a "poor-man's z-stack") of 210 images that is easily delivered and used with ordinary web playback tools. During replay of the sequence, the user can simulate focus by manually manipulating the scrub bar of the video player.

Wright- stained peripheral blood smears were examined and white blood cells photographed in a systematic scan as if performing a 100 cell differential. The cells were not selected but taken in sequence to prevent selection bias and to provide a realistic representation of cells that the students would encounter in practice.

In all, 2693 cell image sequences were collected from 25 cases that were grouped in five complexity levels composed of five cases each. Only the first 100 cells from each case were presented to the student and the others were considered spares for substitution in future applications. Complexity level one represented normal patients while level two represented cases with reactive lymphocytes. Level three represented cases exhibiting the granulocytic maturation series and levels four and five represented chronic and acute leukemic processes.

Computer program description

The basic function of the training software was to present the media, collect and evaluate the student's interpretation and give performance feedback when the case was finished. The prototype computer program was developed using FileMaker Pro 10 Advanced (FileMaker, Inc, Santa Clara, CA). The prototype featured interactive layouts for data and media display and used the FileMaker scripting language for programming interactivity. The programming details are complex and beyond the scope of this paper but the flowchart describes the simple functionality (Figure 1). The resulting software packages and media assets were then installed on seven computers in the student computer lab.

In the background, the prototype software tracked three key usage functions and allowed for password-protected extraction of the usage data. The trackers recorded the number of times that a student began a case, the number of times that they reviewed a missed cell and the number of times that they used the search function.

Students were assigned five cases to complete, three from level two and two from level three and were instructed to submit printed results as proof of completion. The exercise was ungraded but points for completion of the assignment were counted toward the laboratory grade. The software was first demonstrated in the classroom by performing an example case as a group. The optional features, missed cell review and search of cells by cell type, were then demonstrated and discussed in a question and answer period. Students were encouraged to explore the software, to perform extra cases (other than those assigned) and to learn by searching and reviewing galleries of cell types. We wanted to know if the students would enjoy participation and use the tool to master cases beyond those levels that were assigned. The students were allowed one month to use the tool before the usage data was extracted.

Each student's results were analyzed to determine the number of discrepancies from the expert consensus (missed cells) for each case performed. The numbers were then totaled and the students ranked. Item analysis was performed to determine the cells that proved most problematic to the students.

Student evaluations of the tool were requested but not required. The form included questions on the quality and usefulness of the technique. Three open-ended questions allowed for praise, criticism and suggestions on how to improve the tool.

Expert Opinion

Prior to using the tool with students, experts evaluated the cells and submitted data. The expert data collection software was a modified version of the trainer software with additional form fields added to allow for comments and other specific metadata about the cell (e.g. toxic granulation, reactive lymphocyte, etc.). The

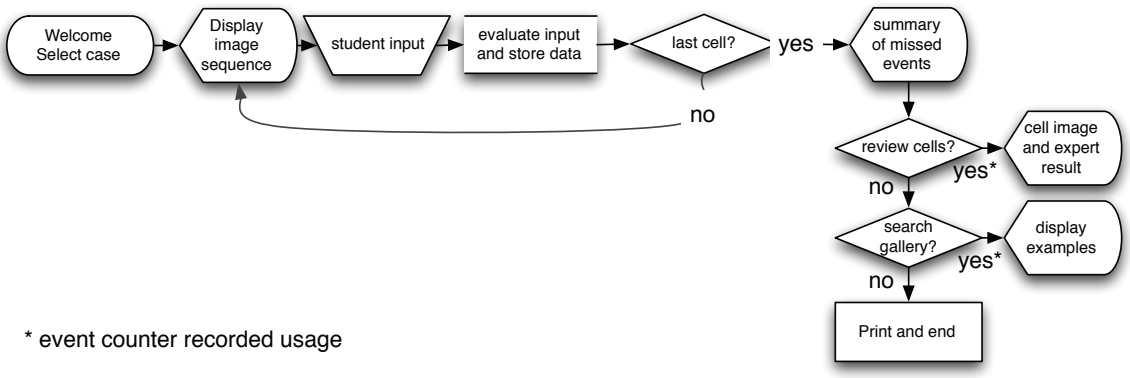

Figure 1. Flowchart of programming steps in the prototype version of the WBC identification trainer.

experts were volunteers and represented hematology instructors from CLS and MLT programs. Twenty-nine teachers were recruited at the national Clinical Laboratory Educator's meeting but only seven participating experts represented programs in the Northeast (2),Midwest (3) and Southeast (2). The expert data was aggregated to determine a consensus answer that was used for evaluating the student's input. A consensus was determined to be the majority opinion when four or more experts agreed on the cell type. Full consensus was determined to be when all experts agreed. When a student chose to review a missed cell, all of the expert opinions and metadata was displayed along with the cell image sequence.

Each expert submission was evaluated to determine the number of discrepancies from the consensus opinion and these were used to rank the experts. Item analysis was also performed on this data to determine the cell types that proved to be problematic to the experts.

RESULTS

The ranking of students based on number of discrepancies showed that they varied in skill in identifying the cells presented by the tool. One student misidentified only 40 cells of the 500 represented in the exercise (8%) but two students exceeded 90 misidentified cells (18%). Figure 2 shows the distribution of student performance expressed by number of discrepancies from the expert consensus.

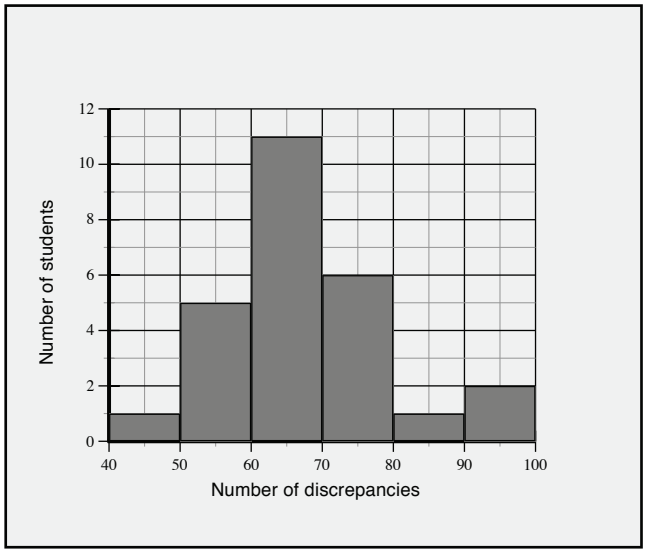

Figure 2. Bar graph showing the number of students (y –axis) by the number of discrepancies from the expert consensus (x-axis).

The most frequent discrepancy in this sample involved identification of large lymphocytes and monocytes but students also frequently mistook monocytes for bands and metamyelocytes. Degranulated basophils were also problematic as some students identified these as segmented neutrophils. A comprehensive profile of problematic cells seen in all 25 cases is not possible since only five cases from levels two and three were assigned in this study. The test sample did not contain any level 1 cases (normal) or any from levels 4 or 5 (containing cases of chronic and acute leukemia).

The usage data from the embedded counters showed 156 starts. All students completed all assigned cases for credit, which would require 130 starts. So the ratio of started cases to assigned cases was 1.2 to 1. On average, each student started cases 6 times to complete the 5 assigned.

The total number of discrepancies for all students was 1738 and the tool tallied 718 requests for cell review, meaning that the students reviewed the missed cells 40% of the time. The tracker on the search function recorded a total of seven searches for all workstations in the one-month usage period.

The activity evaluation was completed by 16 of 26 students (62%). The majority of responses were favorable and the open-ended responses were mostly general in nature but some revealed improvement opportunities. Specifically mentioned was the sharpness of the image, confusion when expert data showed disagreement and the desire to access the tool from home computers and mobile devices.

Expert Results

Sixty percent of cells had full consensus and 1% (18 cells) did not have a consensus reached. Figure 3 illustrates the degree of expert consensus and percent of times that full consensus was not reached by number of outliers.

One expert differed from the consensus opinion only 22 times (0.8%) but two participants differed at more than 10 times that rate (8.9% and 9.7%). The other four participants differed from the consensus approximately 3% of the time. Figure 4 illustrates the number of times opinion differed from the consensus by expert.

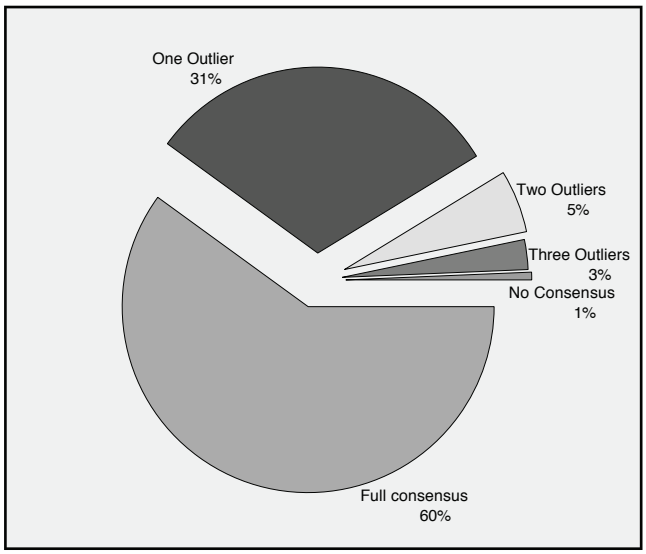

Figure 3. Pie chart demonstrating the percent of agreement between experts interpreting cells with the WBC identification trainer.

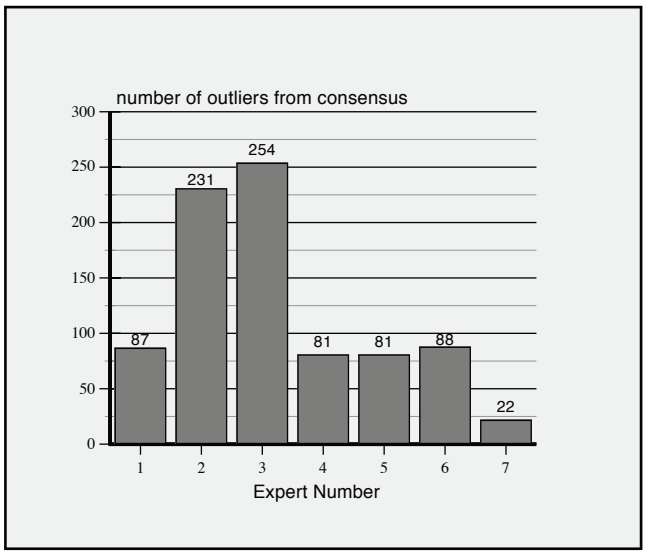

Figure 4. Bar graph indicating the number of discrepancies from the expert group consensus (y-axis) by individual expert (x-axis).

The identification of band versus segmented neutrophil was the most common case failing to get full consensus followed by blast cell identification, lymphocyte/monocyte differentiation and the staging of neutrophil precursors. Figure 5 illustrates the categories of disagreement with percentages.

DISCUSSION

The performance data from both students and experts

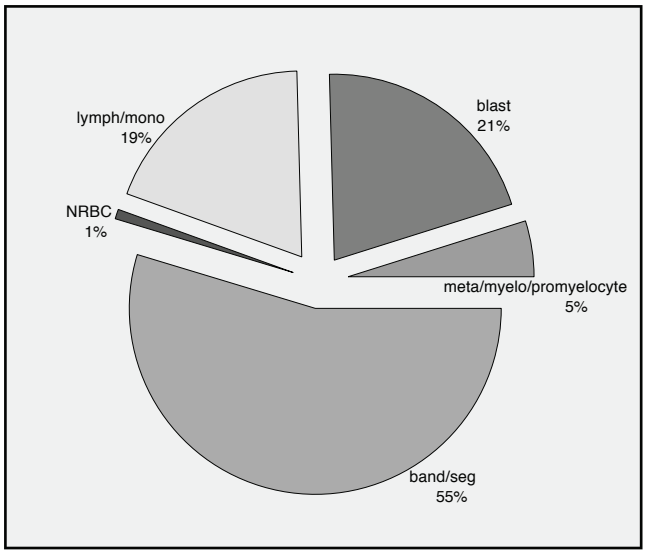

Figure 5. Pie chart demonstrating the categories of cells where the experts disagreed.

suggests that the tool can differentiate users' ability to identify WBCs. Some users show a low number of discrepancies while others exceed these by an order of magnitude. This finding was expected in the student group but somewhat surprising in the expert group.

The usage data indicates that students did not use the tool to master cases more difficult than those assigned. Practice and exploration was determined to be minimal at best because it is reasonable to believe that some extra starts were in fact "do-overs" or "start-overs". We conclude that assignments should be used to direct the student's progress.

Similarly, the relatively low rate of missed-cell review suggests that the review step should be automatic before allowing the student to proceed. This step came at the end of the case, was optional and was only used 40% of the time. It is our desire that the student take a second look at missed cells in light of the expert opinion as a method of calibration to the expert. Also the low number of searches performed, further supports the need to create assignments if searches are deemed desirable to learning. We conclude that the tool as designed is not fun for the students to use.

We are hesitant to over-analyze student evaluations from such a small number of participants but there were a number of negative ratings on image clarity and the written comments indicate a need to improve the image

sharpness over the relative "fuzziness" (as noted by one student) seen in the image sequences. This "fuzziness" is presumed to be due to compression of the image in the movie making process. To address this issue, we now use a hybrid approach. A higher quality static image is initially displayed and the user clicks on the static image to call up the image sequence. We continue to offer the image sequence because some students reported favorably on the technique and we are hopeful that in the future, a technological advance will improve the clarity of the sequence. We want to continue to explore the use of this simple and efficient capture technique.

The student dissatisfaction with seeing the varying expert opinions is also addressed in the new version. Now, cells with more than one disagreeing expert are removed from the library and only the consensus opinion is shown to the student. The original design was to collect 100 cells without selection to eliminate bias and provide real world experience. But now the idea is to eliminate controversy, concentrate only on cells where identification is validated by near full consensus and consider adding back the more complex features as more experience is gained.

The expressed desire to make the tool available at home and on mobile devices is also addressed. The tool is now web based and all modern browsers based on web standards, including mobile browsers, will execute the program.

The new version also provides an assessment-only mode with all feedback steps deactivated but other functions left intact. So the teacher can assign either tutor mode or assessment mode by using a different URL.

CONCLUSIONS AND RECOMMENDATIONS

Weakness in using digital systems have been described including image quality, costs, ability to focus and selection bias.[6] This project explored those weaknesses and weighed those against the reported advantages of image reproduction and distribution at distant sites at different times. The data suggests that the tool can differentiate users at both the novice and expert level, it can identify problematic cell types and it could have potential use in teaching and competency assessment.

The trade-offs considered in tool development are illustrated in this preliminary study and reveal many opportunities to improve the original design. As a desktop application, the tool required installation and limited the access to the computer lab. Too many cases were acquired in the initial design as we naively thought that the students would freely explore the other cases and master higher levels of complexity. We wasted a lot of time (and the time of our experts) to build such a large case library. The library may be useful for future projects. But since we only collected image sequences to build the first library and the new system uses a still image plus the image sequence, we had to start over and collect new media.

For the good parts, the study provided usage data and student opinion to instruct the development of subsequent versions. Also, the skills learned in developing the prototype gave the team confidence to learn the technologies necessary to web development.

With the web version, it is much easier to collect expert data and deploy a single case to students since all media and programs are installed on the web server instead of individual desktop computers. All programming parts are reusable and any updates reach all users automatically. The instructor now sends the assignment link via email and the results are emailed back to the instructor. The instructor can assign increasingly complex cases as the student progresses.

The new tool has proven to be robust thus far and serves as a practical adjunct to our current teaching methods. Item analysis identifies problematic cells and these can be brought to the classroom, projected and discussed. The tool differentiates user skill level and provides a basis from which improvement could be measured. The ability to detect poorer performance offers the potential for teachers (and lab managers) to customize remediation efforts for maximum effectiveness instead of doing one-size-fits-all training.

While the design is intended for hematology, the technique could be adapted to other disciplines such as microbiology and urinalysis where visual recognition skills are needed and where valid expert feedback may be obtained. In addition to use in student assessment, the tool might be used for research applications to answer questions about competency throughout the career of practitioners and to ensure the quality and consistency of faculty in teaching programs.

This study and tool is very limited in addressing questions about microscopic skills. For instance, the simulator does not require microscope setup and since the images are displayed it does not address the searching and finding aspects of blood film examination. It only the addresses the interpret phase and only for the identification of the cell lineage and development stage of the white blood cells. The study does not show that the tool can improve performance but only seeks to establish a practical platform for measurement. Future studies will have to establish improvement to show that the tool has true value. Since all tool users know that they are being assessed, this tool only addresses the cognitive and psychomotor aspects of performance. It does not address the affective aspects necessary to real world performance and this might be better addressed with a study using retrospective review of real work.

In spite of this study's limitations, we believe that improving the skills in WBC identification is worthy of our efforts. To paraphrase Lord Kelvin, "if you can measure it, you can improve it" and while WBC identification is not the only element of peripheral blood film examination, it is a key part. It is reasonable to assume that health care organizations prefer experts to poor performers. Therefore, we conclude that it is reasonable to continue to seek tools that create experts and this type of tool is feasible to implement and worthy of future study.

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Some Basic Points Concerning Meta-Analysis

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ABSTRACT
Multiple studies have been performed on a variety of substances, often producing contradictory results. Meta-analysis has provided a means of evaluating these disparate results, combining them into a summary statistic. Using continuous data for baseline and one sample point, several studies were evaluated to achieve a single result, demonstrating the meta-analysis evaluation process.

ABBREVIATIONS: ANOVA-analysis of variance, H_0-null hypothesis, H_A-alternative hypothesis, FDA-Food and Drug Administration.

INDEX TERMS: Meta-analysis, Statistics, Study results

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In the past, it has been very useful to perform statistical analyses on study data to evaluate the test substance's effectiveness. If done correctly, the analysis can determine if the test substance was effective. Over the years, multiple studies have been performed, evaluating the same substances in the same general ways. What does one do when one study says a substance is effective, but another study says that substance is not effective?

Meta-analysis is a statistical methodology that allows one to evaluate studies conducted at different laboratory test sites, at different time periods, by different scientists, on different test subjects, and combine those results into one study.[1,2] Meta-analysis uses the results gained from a number of different studies as its data points and analyzes them.[11]

Meta-Analysis for Continuous Data

Meta-analysis can evaluate continuous data, binary data, or the correlation among data. The focus in this paper is continuous data, rather than binary or correlational data, as it is used more frequently in scientific fields. Let us take hand disinfectants as an example. Researchers evaluated a product by measuring the baseline sample (pre-product application) and a post-product application sample. A baseline value and a post-application sample divided by standard deviation was used to calculate a D value in this work. It equals:

$$D = \frac{\bar{x}_{BL} - \bar{x}_{sampletime}}{s_{pooled}}$$

where:

D = the dependent variable, which is the difference of the baseline minus the sample time divided by the pooled standard deviation. Each of the D values was the result of one complete study. The baselines were different for each study, so subtracting the post-application sample time from the baseline provided the reduction in microorganisms. This procedure adjusted all the studies, making it possible to compare them directly by their reduction values. (We will discuss dividing the reduction by the pooled standard deviation in the s_{pooled} section.)

$\bar{x}_{sample\ time}$ = the $\log_{10}$ count average of the sample time. The population counts were not linear but exponential. This greatly complicated the statistical model; hence, they were transformed into linear scale, by taking the $\log_{10}$ of the plate count data.

$\bar{x}_{BL}$ = the $\log_{10}$ colony count average of the baseline. The same transformation to a $\log_{10}$ scale was applied to baseline data.

S_{pooled} = this study involved the baseline and the post-application sample time microbial counts on the same subject. The hands were selected for baseline and post-application sample according to the randomization schedule (left hand versus right hand). This was a paired test (the same subject was used for both readings), which made the standard deviation a pooled standard deviation. However, each study had a different standard deviation. So, to adjust the data for easy comparison, the reductions were divided by the standard deviation. The end result was the D value, which informs the reader how many standard deviations the reduction (baseline – post-application sample) is. For example, if the average baseline was 5.00 $\log_{10}$ and the average wash was 3.00 $\log_{10}$, then 5.00 – 3.00 = 2.00 $\log_{10}$. That is, the product reduced the microbial colony counts by 2.00 $\log_{10}$. If the standard deviation was 1.00 $\log_{10}$, the 2.00 = 2.00/1 represented the number of standard deviations to the right the reduction represented. The S_{pooled} formula was:

$$s_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

where:

n_1 = sample size of the baseline data

s_1^2 = variance of the baseline data

n_2 = sample size of the application data

s_2^2 = variance of the application data

Hedge's g Statistic

The D statistic $\left(D = \frac{\bar{x}_{BL} - \bar{x}_{sampletime}}{s_{pooled}} \right)$ was slightly biased in that it over-estimated the differences with small sample sizes. This bias was removed through a simple correction – the Hedges' g calculation.[12]

The Hedges' g statistic was calculated:

$$g = J \times D$$

where:

$J = 1 - \frac{3}{4df - 1}$ (df = degrees of freedom to estimate S_{pooled}). This was the correction factor. The degrees of freedom was the denominator of the standard deviation; $df = (n_1 + n_2 - 2)$.

D = the dependent variable from the outcome of the difference divided by the pooled standard deviation). It represented how large the reduction was in terms of the standard deviation.

The Hedges' g statistic was used in all the calculations.

Fixed or Random Effects

There are two popular statistical models for meta-analysis, fixed and random effects. Recall that in a general statistical model (e.g., Analysis of Variance [ANOVA]), a fixed effects model means that the dependent variables were chosen before a study began. Using the hand disinfectant example – a healthcare personnel handwash with chlorhexidine gluconate as the main ingredient – if a researcher wanted to evaluate the product against the best-selling chlorhexidine gluconate, s/he decided against what product to test. This was a fixed effects model, because the variable (product) was chosen deliberately. On the other hand, if a random product selection was made from all the chlorhexidine gluconate products available, it was randomly selected for comparison.[15]

In meta-analysis, the fixed and random effects mean have completely different meanings.[9] For the fixed effects model, it was assumed that there was one true effect for all studies in the model. In other words, a drug's effect had the same value among all the different studies, and any differences were purely sampling error.

For the random effects model, it was assumed that there was not one true average value for all the studies combined. They were different values.

In summary, the fixed effects model handled the data with these assumptions:

- There was one true effect size for all studies
- All the different effects were actually sampling errors
- Weights were assigned high values for studies

that had high sample sizes,
- Small sample size studies were assigned smaller weights, and
- Weights of study = $\frac{1}{V_y}$, where $V_y = s^2$ = variance

For random effects, they were handled differently:
- There were different true effects sizes, depending upon the study
- The different effects sizes were presumed real, not sampling errors
- Higher weights were not assigned to studies with larger sample sizes,
- Small sample size studies did not get smaller weights, and
- Weights of study = $\frac{1}{V^*_y}$, where $V^*_y = s^2 + \tau^2$, s^2 = variance, τ^2 = within-subject variance

Understanding these effects is very important in meta-analysis, for using them changes the confidence intervals, as well as the grand total value, often dramatically.

The statistics are rather tedious to compute and usually performed by a computer software program, so they will not be discussed further. For a background of how different statistical programs are run, the procedures can be reviewed.[1,3]

Which Model Should Be Used?

There are several thoughts about which model – fixed or random – should be used. The first paradigm is that the main effects of this hand disinfectant example should be random effects, because the studies included were performed at different times by different people using different subjects. The results were expected to be different. The second paradigm states that it makes sense to use fixed effects if two conditions are met: first, if the researcher believed that all the studies were identical; and second, if the goal was to compare a common effects size from identical populations.[3] These two conditions are not common in hand disinfectant studies. First, for these types of studies where media was placed on the hands, the initial population probably varied, providing different baselines among the different studies. However, this was corrected by using the reduction (baseline – post-application sample) value. This part of the *D* value was discussed previously. For studies that used populations of bacteria normally living on the hand surfaces, and the subjects' counts dependence upon time of year, humidity, and temperature, this method may also be used. Think about the many other areas that are studied, and you will probably see similarities.

The second question was "will the sample size be consistent among studies?" Some studies had as few as five subjects, and others had more than 100; so they varied. To be safe, use of the random effects model was suggested.[9]

These two paradigms were not discussed completely. To determine if the fixed or random effects should be used, there are several other valuable tools. For example, a researcher can check if the groups appear homogenous (the same) or heterogeneous (different). This test examines the *Q* values (discussed later). There are also other tests like finding the I^2, values, which is a kind of signal to noise ratio test, and the *T* value is another test, which is the standard deviation of the true effects size.

These were not the only factors of concern with this study, as was discovered later when the subgroups – the application times (30 seconds and 1 minute) – were included in this handwash model. These applications times were consistent no matter what product was tested or by whom. This categorized the subgroup as "fixed effect," which shall be discussed later. However, had the researcher discovered differences in the studies being compared – for example, if the application times varied inconsistently, the model would have been a random effects component.

Importance of Selecting All Studies, Not Just the "Good" Ones

This is a central point in meta-analysis. It is critical to select all the studies that one can find for the evaluation.[3] Otherwise, for example, the results may be skewed in a direction desirable to the researcher. Using the hand disinfectant example, if the researcher selected only those studies that showed the product to be effective and dismissed those that showed it was not, the meta-analysis would have been biased. But how would a reader know this?

When beginning a meta-analysis, the researcher must define a reasonable inclusion/ exclusion criteria list for the studies and publish it with the results. For example, the inclusion criteria identified all studies that used the FDA handwash guidelines[4] for hand disinfectant studies. Notice that these items were not the way "this test was supposed to be run," but the way the analysis was designed. The exclusion criteria consisted of studies with data generation not clearly understood, types of studies using guidelines different from those of the FDA, and studies not performed in a randomized manner. These two areas require much time and consideration for the selection of studies to be used.[5] Table 1 contains the series of studies included in this analysis.

The eight studies in this meta-analysis fit the inclusion/exclusion criteria just presented.

Meta-Analysis
The main statistical test was:

$$H_0: BL^* = W$$
$$HA: BL \neq W$$

* *BL* = Baseline; *W* = Wash

That is, does a significant difference exist between the baseline and the wash (post-application sample)?
The preliminary meta-analysis is displayed in Figure 1, where each of these studies went through an analysis and received a final or grand total score (bottom line). The 95% confidence intervals are also given, with a probability value. The probability value or *p*-value is the probability (that the true Hedges' *g* value was equal to or greater than $x^* \mid H_0$ true) $\leq \alpha$ or level of significance. x^* = Hedges' *g* actually calculated, and the level of significance for this test is $\alpha = 0.05$.

Each of these eight studies achieved a significance of $p < 0.000$. Note the diamond at the bottom of the graph represents the 95% confidence interval of all eight of the tests (3.920 – 8.285). The grand total of the Hedges' $g = 6.102$. The values were synthesized into one value for the entire meta-analysis. The alternative hypothesis was accepted (H_A); the test was significant. The baseline and the post-application samples were different at $\alpha = 0.05$.

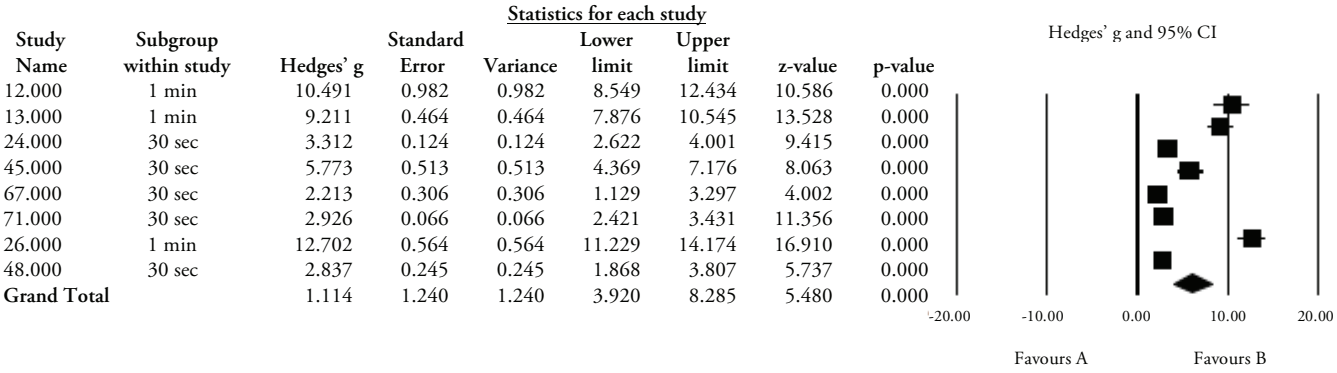
Looking at the graph portion (right-hand side) of Figure 1, differences appeared among the groups, even though this was a random effects study, in which some variation among studies was expected. That is, the Hedge's *g* values did not seem to be homogenous (roughly the same), but instead were heterogeneous (different). So a homogeneity versus heterogeneity test was performed. The random effects model was temporarily changed to a fixed effects model. The test hypotheses were:

H_0: All groups are homogenous, or the same.
H_A: The groups are different (heterogeneous).

Table 1. Study Data

		Baseline			Application of Product		
	Study Name	Group A Mean	Group A Std Dev	Group A Sample Size	Group B Mean	Group B Std Dev	Group B Sample Size
1	12	6.800	0.340	30	3.560	0.265	30
2	13	7.400	0.389	50	3.890	0.367	50
3	24	6.880	0.678	38	4.789	0.567	38
4	45	7.900	0.564	20	4.890	0.452	20
5	67	5.890	0.780	10	3.870	0.959	10
6	71	8.900	0.561	62	6.900	0.780	62
7	26	7.520	0.294	75	3.190	0.379	75
8	48	6.300	0.593	16	4.870	0.362	16

META ANALYSIS



Statistics for each study

Study Name	Subgroup within study	Hedges' g	Standard Error	Variance	Lower limit	Upper limit	z-value	p-value
12.000	1 min	10.491	0.982	0.982	8.549	12.434	10.586	0.000
13.000	1 min	9.211	0.464	0.464	7.876	10.545	13.528	0.000
24.000	30 sec	3.312	0.124	0.124	2.622	4.001	9.415	0.000
45.000	30 sec	5.773	0.513	0.513	4.369	7.176	8.063	0.000
67.000	30 sec	2.213	0.306	0.306	1.129	3.297	4.002	0.000
71.000	30 sec	2.926	0.066	0.066	2.421	3.431	11.356	0.000
26.000	1 min	12.702	0.564	0.564	11.229	14.174	16.910	0.000
48.000	30 sec	2.837	0.245	0.245	1.868	3.807	5.737	0.000
Grand Total		1.114	1.240	1.240	3.920	8.285	5.480	0.000

Figure 1. Meta-Analysis

The *Q* value is a measure of the weighted squared deviations. If the *Q* value was quite large (*Q* > *degrees of freedom* {*df*}), the study had greater deviation (*Q*) than was expected. For this analysis, the heterogeniety test (*Q* value) was 278.472, with $k - 1$ or $8 - 1 = 7$ degrees of freedom; *k* is the number of studies evaluated.

Q value	–	degrees of freedom	=	final value
278.472	–	7	=	271.472

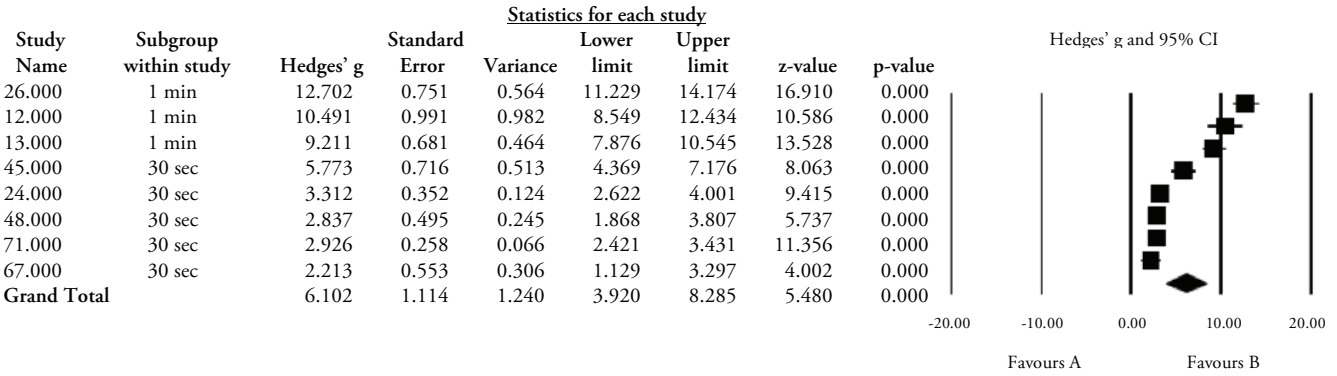
Using the Chi Square test with $k - 1$ degrees of freedom and checking 271.472, a significance of less than 0.05 was achieved ($p < 0.000$). The studies were not homogenous. This *Q* value was too large to ignore.

Viewing the data in Figure 1, study names, the study numbers 12, 13, and 26 were different from the other studies in that they appeared to be much more effective.

To get a clearer picture of this and determine the cause, the data were rearranged from high to low and reviewed (Figure 2).

Going back to the original studies to determine if the products were different or if the application times were longer, a difference was noted. It was discovered there were two product application times, 1 minute and 30 seconds, that were not noted at first. A subgroup (time of wash, either 1 minute or 30 seconds) was then included in the model. Then the temporary fixed model was changed back to a random effects model. If these

META ANALYSIS



Statistics for each study

Study Name	Subgroup within study	Hedges' g	Standard Error	Variance	Lower limit	Upper limit	z-value	p-value
26.000	1 min	12.702	0.751	0.564	11.229	14.174	16.910	0.000
12.000	1 min	10.491	0.991	0.982	8.549	12.434	10.586	0.000
13.000	1 min	9.211	0.681	0.464	7.876	10.545	13.528	0.000
45.000	30 sec	5.773	0.716	0.513	4.369	7.176	8.063	0.000
24.000	30 sec	3.312	0.352	0.124	2.622	4.001	9.415	0.000
48.000	30 sec	2.837	0.495	0.245	1.868	3.807	5.737	0.000
71.000	30 sec	2.926	0.258	0.066	2.421	3.431	11.356	0.000
67.000	30 sec	2.213	0.553	0.306	1.129	3.297	4.002	0.000
Grand Total		6.102	1.114	1.240	3.920	8.285	5.480	0.000

Figure 2. Meta-Analysis Table (High to Low Arrangement)

two levels were not different times, but one time, or were not caused by anything known, they would be discussed in the report and reported as one factor, not two, in a random design.

The Final Model

The final model was composed of two factors: 1) the eight different studies, and 2) the two time intervals. This was a random effects model for the various studies included, which had subgrouped times (1 minute and 30 seconds) embedded in them. The times were fixed effects. This provided a "mixed effects" model. The model selected was also 1) an analysis across levels of the two subgroups, and 2) a comparison of the effects of these subgroups.[10] This study had a common variance that was pooled. Figure 3 presents these data.

There were two sub-analyses occurring in this table. The first (summarized by the first diamond) was for a one-minute application, which provided a Hedges' *g* summary of 10.784 ($p < 0.000$). This was highly significant. There was also a 30-second application. This was not as effective as the one-minute application, but it, too, was very effective. It is summarized by the second diamond, a Hedges' *g* summary statistic was 3.317 ($p < 0.000$). The 30-second and one-minute applications were combined into an overall grand total Hedges' *g* statistic (the third diamond), which was 5.607 ($p < 0.000$).

Notice that there was still heterogeneity within these two times at the 0.05 level. Studies 13 and 26 were different from each other for the 1-minute application. Study 45 was different from all the other 30-second evaluations. They were not compared for homogeneity, because there was no indication they were handled differently. It was assumed that there was much variability among the studies. Therefore, they remained in the random effects model.

To formally test the 30-second and 1-minute application times, examine the two 95% confidence levels: 30 seconds = 2.378 – 4.258, and 1 minute = 9.372 – 12.197. The 30-second and 1-minute confidence intervals did not overlap, so they were different.

In summary, for the hand disinfectant data, the results indicated the product was effective at 30 seconds, but it killed many more bacteria when applied for one minute.

Looking for Bias in the Study

If the studies were all-inclusive in this analysis, then there would be no need to look for bias; however, it was unknown whether this occurred. There were two opportunities for bias to occur. The first, already discussed to some degree, was that studies opposed to the researcher's beliefs were eliminated. For example, a researcher may have chosen only the studies that showed their product superior to others.[3] To this end, significant studies were evaluated, and insignificant ones were not included in the evaluation. This is a major problem in meta-analysis.[1,2,3] The second case was that contradictory studies may not have been published. For example, very small studies or studies showing no effects

META ANALYSIS

Group by Subgroup within study		Study Name	Subgroup within study	Hedges' g	Standard Error	Variance	Statistics for each study: Lower limit	Upper limit	z-value	p-value
1 min		12.000	1 min	10.491	0.991	0.982	8.549	12.434	10.586	0.000
1 min	1 min	13.000	1 min	9.211	0.681	0.464	7.876	10.545	13.528	0.000
1 min		26.000	1 min	12.702	0.751	0.564	11.229	14.174	16.910	0.000
1 min	Total(1 min)		30 sec	10.784	0.721	0.519	9.372	12.197	14.965	0.000
30 sec		24.000	30 sec	3.312	0.352	0.124	2.622	4.001	9.415	0.000
30 sec		45.000	30 sec	5.773	0.716	0.513	4.369	7.176	8.063	0.000
30 sec	30 sec	48.000	30 sec	2.837	0.495	0.245	1.868	3.807	5.737	0.000
30 sec		67.000	30 sec	2.213	0.553	0.306	1.129	3.297	4.002	0.000
30 sec		71.000	30 sec	2.926	0.258	0.068	2.421	3.431	11.356	0.000
30 sec	Total (30 sec)			3.317	0.479	0.230	2.378	4.258	6.922	0.000
Overall	**Grand Total**			5.607	0.399	0.159	4.824	6.389	14.050	0.000

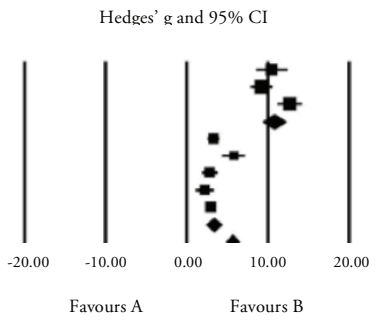


Figure 3. Meta-Analysis: Two Time Points (30 second and 1 minute)

are rarely published.[10] Studies that demonstrate large differences are more likely to be published than studies that do not. Both of these situations represent a potential bias in the meta-analysis.

Because bias cannot be avoided with certainty, its potential is assessed by formulating a few questions:

1. Is there evidence of bias?
2. Is it possible that the entire main effect is due to an artifact of bias?
3. How much impact of bias is present?

We simply do not know these answers, yet. The Cochrane Collaboration[6] has published the results of over 3700 meta-analyses and is a good place for the researcher to begin. It did not have any studies relevant to evaluating topical antimicrobials the way the FDA expects them to be evaluated. There were several studies listed that compared the incidence of disease relative to hand-washing, but this did not coincide with the design of this meta-analysis.

A good place to look for bias in this study was with a funnel plot,[16] which appears as funnel-shaped, or a graph composed of standard error versus the Hedges' *g* statistic (Figure 4). Generally, the smaller the study, the larger the standard error. The standard error is larger in a small study because the values in the numerator are divided by a smaller number in the denominator. This is opposed to a larger *n*, for larger sample sizes in a study, which give a smaller standard error. The funnel shape is caused by ordering the standard errors of the study. For this analysis, the smaller studies with larger standard error were plotted in the bottom portion of the graph; the larger studies with smaller standard error in the top portion (Figure 4).

It is similar to Exploratory Data Analysis (EDA), which, in general statistics, examines the data distribution.[13,14] Looking at a stem-leaf display, a researcher can see if the data fit a normal distribution.[14] Non-biased data would look like Figure 5A. If the data were biased; however, it could be seen that the lower values were removed (Figure 5B). The distribution looks abnormal on the stem-leaf display.

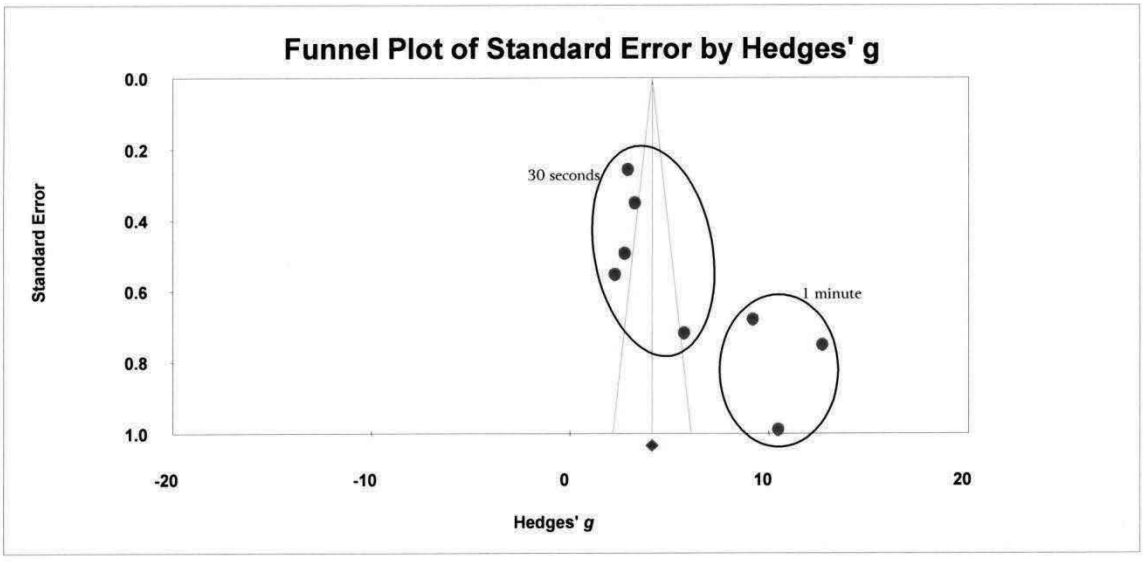


Figure 4. Funnel Plot

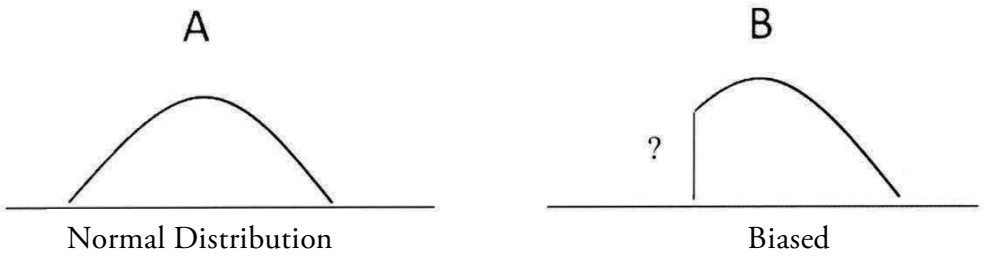


Figure 5. Stem-Leaf Distributions

In meta-analysis, the funnel plot serves a similar condition. In this study, however, there was a problem: two different time frames. The 30-second and 1-minute times were initially separated and two different funnel plots generated. However, the studies were limited to only three data points for the 1-minute time and only five for the 30-second time. This was not enough data to detect a bias if one existed, so the study remained undivided.

It was apparent that the three highest Hedges' *g* studies were performed at one-minute application times instead of 30-second application times. As a result, they pulled the Hedges' *g* to the right (to a higher Hedges' *g*). Another statistic, Duval and Tweedie's Trim and Fill Statistic,[17] used an iterative procedure to remove the most extreme studies by presenting mirror image of the most extreme data points in the graph (Figure 6). Using the Duval and Tweedie's trim and fill statistic showed the mirror image of the two most weighted studies as neutralized. It essentially canceled the two studies with high values by presenting a mirror image of them on the graph. Figure 6 shows the effects.

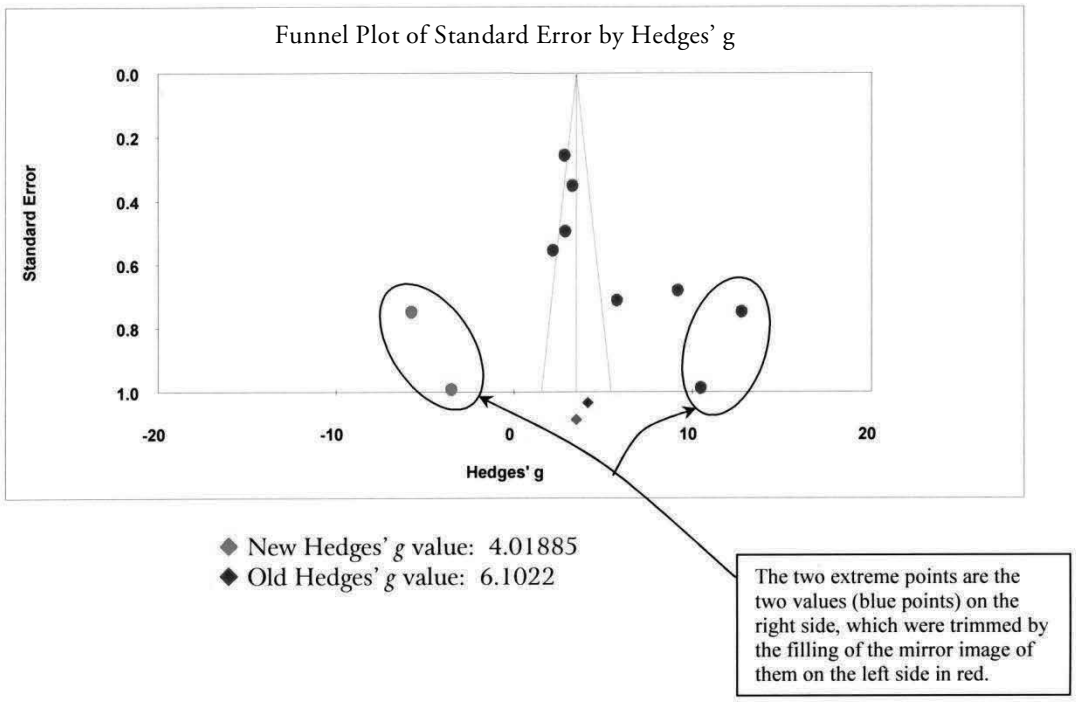


Figure 6. Funnel Plot of Standard Error by Hedges' *g* Using the Duval and Tweedie's Trim and Fill Statistic.

The actual value of the point estimate and the 95% confidence interval are shown in Table 2. The average grand total point went from 6.10220 to 4.01885. The upper and lower confidence intervals were also moved to the left (from 3.91971 – 8.28468 to 1.56302 – 6.47467) but were still significant, because zero was not included in the confidence interval.

Table 2. Duval and Tweedie's Trim and Fill

			Random Effects	
	Studies Trimmed	**Point Estimate**	**Lower Limit**	**Upper Limit**
Observed values		6.10220	3.91971	8.28468
Adjusted Values	2	4.01885	1.56302	6.47467

Note that this is a misrepresentation of this study, for three studies were performed at one-minute application times and five were performed at 30 seconds. At worst, the study results continue to be significant, even though the average effect has moved to the left. The three questions were then addressed. It was not known if bias was present, but there was no evidence of bias. It was possible that the main effect was due to bias, but if there was any, it was inconsequential; the product was still significant.

Conclusion

A researcher should present the data relative to the readers' comprehension, and remember that most readers are not statisticians. The key questions will be "what is easier for readers to understand?" and "How can data best be presented to them?" Meta-analysis allows one to integrate the results of various studies to achieve comprehensive understanding of the studies performed.

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Anticoagulant Therapy Overview

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LEARNING OBJECTIVES

1. Recount the history of heparin, Coumadin, and hirudin
2. Summarize the function of the anti-Xa drugs, vitamin K antagonists, and direct thrombin inhibitors.
3. List two oral anticoagulants that have been cleared by the US FDA since 2009.

ABBREVIATIONS: APTT or PTT-activated partial thromboplastin time; CAD - coronary artery disease; DTI - direct thrombin inhibitor; DVT - deep venous thrombosis; FDA - US Food and Drug Administration; HIT-heparin-induced thrombocytopenia with thrombosis; INR - international normalized ratio; LMWH - low molecular weight heparin; PE - pulmonary embolism; PT - prothrombin time; RI - reference interval; RUO - research use only; TPA - tissue plasminogen activator; UFH - unfractionated heparin; VKORC - vitamin K epoxide reductase; VKA - vitamin K antagonist; VTE - venous thromboembolism.

INDEX TERMS: Anticoagulants, heparin, fondaparinux, rivaroxaban, apixaban, Coumadin, warfarin, lepirudin, argatroban, bivalirudin, dabigatran, prothrombin time, activated partial thromboplastin time, anti-Xa heparin assay, atrial fibrillation, thrombosis, thromboembolic disease, coronary artery disease.

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Anticoagulants do not thin the blood, even though we call them "blood thinners."[1] Blood viscosity is a function of hematocrit and, in leukemia, white blood cell burden, but not plasma protein concentration or enzyme activity. Although thrombolytic "clotbusters" like tissue plasminogen activator (TPA) or streptokinase can dissolve clots, anticoagulants cannot, another popular misconception. Anticoagulants can only prevent thrombus formation or extension by reducing plasma thrombin activity, the basis for their effectiveness. Though this may seem like a limitation, anticoagulants save many lives and promote recovery following a thrombotic (clotting) event.[2]

Anticoagulant treatment is either prophylactic or therapeutic. Prophylactic anticoagulation includes the prevention of ischemic stroke in people with chronic atrial fibrillation or artificial heart valves, or the prevention of venous thromboembolic (VTE) disease in the form of deep venous thrombosis (DVT, clots in major leg veins) or pulmonary emboli (PE, clots in lung vasculature), subsequent to orthopedic surgery, neurosurgery, or during a complicated pregnancy.[3] Therapeutic anticoagulation, which usually implies higher doses than prophylactic anticoagulation, is used in patients with current VTE disease or those with coronary artery disease (CAD) complicated by cardiac insufficiency.[4]

Anticoagulants are a subset of a family of drugs called antithrombotics, which include the oral antiplatelet drugs such as aspirin and clopidogrel (Plavix); the intravenous antiplatelet membrane glycoprotein receptor drugs, abciximab, eptifibatide, and tirofiban; and the thrombolytics.

This series does not address the antiplatelet drugs or the thrombolytics. In this series we first address the anti-Xa anticoagulants, heparin and its analogues; next, the historical mainstay Coumadin, our only vitamin K antagonist (VKA); and last, the direct thrombin inhibitors (DTIs), represented by their original synthetic hirudin (lepirudin), analogue of a leech saliva component, now discontinued; bivalirudin, an

abbreviated form of lepirudin; argatroban; and the first oral DTI, dabigatran, released in 2010 for stroke prevention in chronic atrial fibrillation.

Heparin and The Parenteral Anti-Xa Anticoagulants

In 1916, Jay McLean and William Henry Howell extracted heparin, the first anticoagulant, from dog liver.[5] They recognized its anticoagulant properties and derived its name from its liver origin. In 1935, Erik Jorpe of the Karolinska Institut, Stockholm, accurately described heparin's chemical structure, whereupon the University of Toronto Connaught Medical Research Laboratories produced a heparin salt safe for human therapy. Heparin was cleared as a therapeutic by the newly formed United States Food and Drug Administration (FDA) in 1937.

Heparin, now called standard or unfractionated heparin (UFH), is a linear glycosaminoglycan composed of an average of sixty sugar molecules, one of the most negatively charged molecules in nature. UFH mimics endothelial cell heparan sulfate, is extracted from porcine stomach and intestinal linings, and since 1937 it has been administered intravenously to successfully prevent and treat both arterial and venous thrombosis. The pharmacokinetics of UFH are idiosyncratic, thus heparin therapy requires frequent laboratory monitoring until it is discontinued.[6] This is because UFH differentially binds patients' endothelial cell surface molecules and a variety of patients' plasma proteins.

In 1993 the US FDA cleared low molecular weight heparin (LMWH), another glycosaminoglycan, which is derived from UFH through enzymatic or chemical depolymerization. LMWH's predictable dose-response properties make it safer and easier to administer than UFH. LMWH, injected subcutaneously, is available for either prophylaxis or treatment of venous or arterial thrombosis, and requires no recurrent laboratory monitoring. The most commonly prescribed brand of LMWH in the US is Lovenox®, generic name enoxaparin, marketed by Aventis, Inc.

Pharmaceutical manufacturers prefer to develop anticoagulants that require no laboratory monitoring, a significant convenience for the patient and an obvious marketing advantage. Consequently, drug monitoring is not included in clinical trials. LMWH was the first of the anticoagulants possessing stable pharmacokinetics, and when it was released, laboratories had no FDA-cleared means to measure its plasma concentration. Although we need not regularly monitor LMWH as we do its more dangerous parent, UFH, we often must *test* for its plasma concentration in anyone who possesses a coagulopathy or osmotic imbalance. These include pregnancy, patients with renal or hepatic disease, cancer, diabetes, or a previous thrombotic event; the morbidly obese, the exceptionally slender, or children. We also required a means to check for compliance or to identify the cause of hemorrhage for emergency department patients unable to name their drug. The *in vitro* diagnostics (IVD) industry scrambled to develop an effective laboratory test, the anti-Xa heparin assay, which is now in common use, because the traditional assay that was used to monitor UFH, the activated partial thromboplastin time (APTT, PTT) was insensitive. Laboratory directors and IVD manufacturers are repeatedly challenged to find clinically effective means for testing new anticoagulants as they are released, and are currently scrambling for ways to measure the new anticoagulants dabigatran and rivaroxaban.

In 2001, the US FDA cleared danaparoid sodium, a judiciously titrated mixture of heparan sulfate, dermatan sulfate, and chondroitin sulfate. Danaparoid sodium is the first synthetic form of heparin. It has never been successfully marketed in the United States, but remains available in Northern Europe as Orgaran®, marketed by Schering-Plough, Inc.

Fondaparinux, a synthetic that mimics a specific heparin pentasaccharide sequence and is employed for the same indications as LMWH, was released in 2001. Fondaparinux is marketed as Arixtra® by GlaxoSmithKline and has better efficacy and safety than LMWH. A generic form of fondaparinux was cleared in 2011 after Glaxo's patent expired. UFH, LMWH, danaparoid, and fondaparinux all raise the effect of plasma antithrombin upon the serine proteases of the coagulation cascade, especially thrombin (IIa) and activated coagulation factor X, or Xa.

Rivaroxaban, the first of the oral direct anti-Xa anticoagulants, was approved for the prevention of stroke in patients with atrial fibrillation in November, 2011, and apixaban, the second oral direct anti-Xa anticoagulant was cleared for the same purpose in

December, 2012. Rivaroxaban and apixaban act directly to inhibit Xa activity, bypassing antithrombin. Rivaroxaban is marketed as Xarelto® by Janssen Pharmaceuticals, Inc, and apixaban is marketed as Eliquis® by Bristol-Myers-Squibb, once cleared. Both can be monitored using a modification of the anti-Xa heparin assay, however the modification requires new FDA clearance and is currently under review.

Read about the means for laboratory monitoring of UFH, LMWH, rivaroxaban, and apixaban in the accompanying article, Monitoring the Anti-Xa Anticoagulants, from Heparin to Eliquis.

Vitamin K Antagonist: Coumadin

The familiar history of the vitamin K antagonist (VKA) Coumadin begins in 1921 when Canadian veterinary pathologist Frank Schofield proved that an epidemic of fatal cattle hemorrhages in the Northwest US and Southwest Canada was caused by fungus-infected (spoiled) sweet clover, a component of silage. In 1933, Dr. Karl Link, University of Wisconsin, assigned graduate students the task of deriving and synthesizing the component responsible for the loss of cattle, and in 1940, Wisconsin graduate Harold Campbell first characterized dicoumarol, a synthetic analogue of coumarin, the compound that produces the pleasant odor of newly mowed hay or grass. Spoiled sweet clover produces 4-hydroxycoumarin, the anticoagulant responsible for the bleeding disorder in cattle, mimicked by the synthetic dicoumarol.

Link and his chemists continued to develop dicoumarol, and by 1948 were awarded a patent for warfarin, which soon became popular as a commercial rodent poison. Warfarin is named for the Wisconsin Alumni Research Foundation (WARF), which funded Link's research and held the patent.

In 1951 a soldier attempted suicide by ingesting rat poison, and his symptoms were reversed using vitamin K. By 1954 the medical version of warfarin, Coumadin, was released by the US FDA, and in 1955 the drug gained fame as President Dwight D. Eisenhower was prescribed Coumadin following a heart attack. Coumadin was intended to prevent recurrence of myocardial infarction and was used in this capacity until approximately 2000 when it was largely replaced by more effective antiplatelet therapy.

The term "vitamin K antagonist" is a biochemical misnomer. Instead, Coumadin inhibits the liver enzyme vitamin K epoxide reductase (VKORC) that normally reduces oxidized vitamin K. Reduced vitamin K catalyzes the γ-carboxylation of coagulation factors II (prothrombin), VII, IX, and X and coagulation control proteins C, S, and Z. Vitamin K becomes oxidized in the process of γ-carboxylation, and requires VKORC to become reduced and reenter the reaction. Inhibited by Coumadin, the liver produces ineffective "des-carboxy" forms of II, VII, IX, X, C, S, and Z, thereby reducing thrombin activity and thrombus formation.

Coumadin is the most popular, yet the most dangerous of the anticoagulants. It must be monitored at least every four weeks for the duration of therapy, often lifelong. The clinical laboratory offers the prothrombin time (PT), whose results in seconds are exponentially modified by the international sensitivity index, a measure of thromboplastin reagent sensitivity, to generate the international normalized ratio (INR).[7] An overdose produces hemorrhage, inadequate dosing leads to a repeat adverse thrombotic event, and the therapeutic range, INR 2.0–3.0, is narrow. The indications, mechanism of action, dosing, monitoring, and limitations of Coumadin are discussed in the accompanying article, Coumadin, the Original Oral Anticoagulant.

Direct Thrombin Inhibitors

The "youngest" class of anticoagulants, direct thrombin inhibitors (DTIs) bind and inhibit thrombin without involving any other coagulation protein.[8] Hirudin, a component of *Hirudo medicinalis* (medicinal leech) saliva, was first described as an anticoagulant by Haycraft way back in 1884, was chemically characterized in 1976, and synthesized as lepirudin by Berlex, Inc in the 1990s. Lepirudin (Refludan®), a 65 amino acid polypeptide, was cleared by the FDA in 1998. It was indicated as a substitute for UFH in patients who had developed heparin-induced thrombocytopenia with thrombosis (HITT, HIT).[9] Largely replaced by the oligopeptide bivalirudin (Angiomax®), released in 2006, lepirudin's European producer terminated its production on May 31, 2012.

A more popular DTI, GlaxoSmithKline's small molecule Argatroban®, was released in 2002 to function, like lepirudin, as a heparin substitute in HIT.

Argatroban and bivalirudin are administered intravenously and are used in conjunction with aspirin and clopidogrel.

In 2004 the FDA rejected ximelagatran (AstraZenaca's Exanta®), an oral DTI with considerable promise.[10] Exanta would have been the eagerly awaited first new oral anticoagulant since Coumadin, however it failed to clear because it caused elevated liver enzymes in 5–10% of clinical trial subjects. Although it had cleared in Northern Europe, the manufacturer withdrew the drug in 2006 as it appeared to produce chronic liver disease in a few patients.

In 2010, the US FDA cleared Boehringer Ingelheim's oral DTI, dabigatran etexilate (Pradaxa®), the first successful oral anticoagulant since Coumadin. Pradaxa is currently indicated for prevention of ischemic stroke in atrial fibrillation, although it may later be cleared for VTE prophylaxis in orthopedic surgery and neurosurgery. As was the case with enoxaparin and fondaparinux, no laboratory tests are currently cleared to detect and measure DTI plasma concentrations, hence IVD developers are feverishly conducting clinical trials for a variety of potential assays, as described in our accompanying article, Monitoring the Direct Thrombin Inhibitors.

Medical laboratory science is ever changing, and nowhere as rapidly as in the field of anticoagulant therapy. After 50 years using the same two anticoagulants, clinicians now have a surfeit of choices, with several more in the works. Perhaps by the time these articles are published, apixaban is recently cleared for prophylaxis, and perhaps by the time these articles are published, some new DTI or anti-Xa anticoagulant will have reached the FDA for review. Further, assays currently categorized as research use only (RUO) will be available in automated or kit form, making it possible for acute care facilities to conveniently assay the new drugs.

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Monitoring Coumadin-The Original Oral Anticoagulant

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LEARNING OBJECTIVES

1. Discuss the use of the prothrombin time (PT)/international normalized ratio (INR) for monitoring subjects on the oral anticoagulant Coumadin.

2. Be aware of the relevance of clinical and genetic data (CYP2C9, VKORC1) in predicting Coumadin dosing in subjects requiring different INR ranges.

3. Be knowledgeable of the chromogenic factor X (CFX) assay for monitoring Coumadin in subjects with lupus anticoagulants, transitioning subjects to Coumadin from direct thrombin inhibitors and for accurately following patients with elevated INRs.

ABBREVIATIONS: CFX - chromogenic factor X; CLSI - Clinical and Laboratory Standards Institute; CYP2C9 - cytochrome P450; DTI - direct thrombin inhibitor; FII - coagulation factor II (prothrombin); FX - coagulation factor X; GMNPT - geometric mean normal prothrombin time; INR - international normalized ratio; IRP - international reference preparation; ISI - international sensitivity index; LA - lupus anticoagulant; OAT - oral anticoagulation therapy; PGX - pharmacogenetics; PST - patient self-testing; PT - prothrombin time; TTR - time in therapeutic range; VKORC1 - vitamin K epoxide reductase complex subunit 1; WHO - World Health Organization

INDEX TERMS: Coumadin, international normalized ratio, genetic data, chromogenic factor X

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The prothrombin time (PT) has been the primary screening test for the laboratory evaluation of patients with an acquired or inherited coagulation factor deficiency of what was originally known as the extrinsic or common pathway of coagulation. It is used in conjunction with the international normalized ratio (INR) to monitor oral anticoagulant therapy (OAT) subjects on the oral anticoagulant, Coumadin.[1]

Tissue factor (TF) *in vivo* activates the coagulation cascade through the formation of the TF/FVIIa complex. The PT adds a TF/calcium mixture to citrated platelet-poor-plasma (PPP) *in vitro* to obtain the time for clot formation. There are many variables such as proper specimen collection, multiple reagent/instrument combinations and, most importantly, the sources of thromboplastin, usually rabbit and recombinant tissue factor, selected for the assay.[1,2]

The INR and Local INR Calibration

The commercial thromboplastins vary widely in their sensitivities to Coumadin. Instrumentation differences (photo-optic or mechanical clot detection) may also play a clinically significant role in the performance of the PT/INR assay. With so many variables, providers treating subjects on OAT can be confused when comparing patient results from a number of laboratories using different reagent/instrument combinations. A facility that uses a high sensitivity thromboplastin will generate longer PT results than an assay that uses a low sensitivity thromboplastin. Therefore a subject on OAT may have a PT of 14 seconds with a low sensitivity reagent or 18 seconds with a more sensitive thromboplastin. Hence a subject monitored with insensitive thromboplastins would appear to require a higher dosage of Coumadin to result in an appropriate

prothrombin time ratio.[1,2]

In 1977, the World Health Organization (WHO) realized the problems associated with comparing PTs that are performed with different reagents and introduced a standardized thromboplastin that became their international reference preparation (IRP).[2] In 1983 the WHO described a model for standardizing the PT based on a method in which the PT value is reported as an INR. The INR is the PT that would be obtained if the assay were performed using a WHO primary reference with an international sensitivity index (ISI) value of 1.0. The ISI compares the sensitivity of a known thromboplastin to an international reference plasma calibrated by the manufacturer using the WHO reference plasma. The ISI is an indicator of the thromboplastin reagent's sensitivity to factor deficiencies in comparison to a known standard. The patient's PT, the geometric mean PT of the reference interval and ISI are required to calculate the INR. The outcome of this calculation is used to treat subjects on Coumadin to prevent thrombosis.[3,4]

The formula for calculating the INR is as follows:[5]

$$INR = \left(\frac{\text{Patient PT (sec)}}{\text{MN PT (sec)}} \right)^{ISI}$$

The INR compensates for differing reagent and instrument combinations. Even with different ISI values, the patient values can be theoretically compared using the calculated INR thus allowing a better and more normalized regulation of OAT.

Over the years those reviewing the INR computation have examined the assignments of the ISI values, instrument methods, and calculation errors. A wrong assignment of the ISI may result in deadly inaccurate values. These differences could result in inappropriate dosing of patients on OAT leading to bleeding or repeat thrombosis.[2, 6-8]

INR variables have led to recommendations to locally calibrate the ISI with each laboratory reagent and instrument combination using commercial INR calibrators. A recent publication discusses local ISI validation and calibration.[4] The author uses FDA-cleared kits from vendors who furnish certified plasmas, techniques and examples of data calculations. The procedure can be performed in a laboratory that currently performs a PT/INR. The validation requires three days of testing on certified plasmas (INRs from 1.5–4.5) and calculations that are performed locally or using an automated vendor template. This local calibration should be performed any time a new reagent/instrument or lot of reagents is changed for performing a PT/INR assay in the facility. Each laboratory should be responsible for determining the local ISI, as the manufacturer cannot duplicate the performance of the local test set-up or the proficiency of the employees performing the testing for OAT. The necessity for local calibration of the ISI is imperative and should be performed following strict guidelines from the Clinical and Laboratory Standards Institute (CLSI).[4,5]

By reporting a locally calibrated INR, each facility is using a standardized unit related to the WHO standards. The INR provides the opportunity to use a common unit for defining OAT therapeutic ranges. Clinicians treating subjects being monitored for OAT for arterial or venous thromboembolism target an INR of 2.0–3.0. The target range for a patient with a mechanical heart valve may be 2.5–3.5.[9]

Monitoring Coumadin Using POC Instrumentation

Point of care (POC) instruments are available that are able to determine patients' INR from capillary blood. POC reagent cartridges contain only thromboplastin since the specimen is not citrated blood. These instruments are primarily used in anticoagulation clinics, physician offices, or by OAT patients using home-care devices. However, there are no INR calibrators available to locally calibrate the ISI on POC devices and they should be compared to the main facility's reference method when confirming critical values.

OAT efficacy is influenced by the patient's diet, supplement interactions, body mass index, age, gender and liver functions. The INR should be carefully followed with regard to the subject's drug regimen, wellness and dietary changes.[10] An INR result is not meaningful in subjects who are not on OAT.

When to test and time in therapeutic range (TTR) of 2.0–3.0 are extremely important to prevent the risks of a bleeding event or stroke. Dr. Jack Ansell, MD, international anticoagulation expert and the driving

force behind the Anticoagulation Forum recently presented the results of the Self-Testing Analysis Based on Long-Term Experience (STABLE) study at the March 2012 American College of Cardiology Summit. This study looked at 29,500 U.S. based, real-world patient self-testers (PST) for more than two years and found that PST using POC testing as part of a comprehensive support service had a significantly higher percentage of TTR (74.0%) when they tested weekly. They also benefited from reductions in time spent in the critical INR ranges (INR <1.5 or >5.0), which may triple prevalence of stroke or major bleeding events, respectively. This study states, "by applying more frequent self testing to warfarin therapy, one can optimize safety and efficacy." The TTR demonstrated in this study surpassed that of other, well-designed clinical trials, which included the 2,922 patient, VA Cooperative THINRS trial (TTR=66.2%), presented at American Heart Association meeting in 2008. These data come just before the American College of Chest Physicians (ACCP) released clinical evidence in support of patient POC testing over usual outpatient INR testing.[11, 12]

Conversely, another study of 250 subjects monitored over a year evaluated Coumadin dose assessment every 4 weeks versus every 12 weeks in patients with stable INR.[13] They investigated whether assessment of warfarin dosing every 12 weeks was as safe as every 4 weeks. They monitored subjects whose dosing was unchanged for at least six months. The TTR was 74.1% in the 4-week group compared with 71.6% in the 12-week group. The authors' conclusion was that assessment of warfarin dosing every 12 weeks seems to be as safe as testing every 4 weeks. The testing was performed in a single anticoagulation clinic in which participants coordinated with clinic personnel every four weeks.[13] This is a smaller study than previously discussed but it demonstrates how a well-controlled patient population can be maintained in the TTR with fewer testing times. Even though the data conflict with the previous study it seems that monitoring patients frequently through special anticoagulation clinics seems to keep individuals in the TTR better than a single physician setting.

Screening for Mutations Prior to Coumadin Therapy

Genetic testing for OAT was discussed starting in the early 2000's.[14] The FDA even commented on the subject (Critical Path Initiative: Warfarin dosing on July 27, 2007, www.fda.gov/oc/initiatives/criticalpath/warfarin.html) stating that patients would greatly benefit from genetic testing to enable warfarin dosing.[15] However, as yet this is not a frequently-requested test.

OAT dosing is difficult for a number of reasons. The narrow therapeutic window and wide range of individual response can be influenced by age, gender, diet, medication interactions, disease state, and variations in genetic polymorphisms.[16] Differences in a gene of the cytochrome oxidase-reductase system (CYP) P450, 2C9 gene CYPC2C19 and in the vitamin K epoxide reductase (VKOR) C1 (VKORC1) are associated with slowed Coumadin metabolism. A subject's ability to respond avidly to Coumadin is controlled by the VKORC1 gene.[14] This gene controls the site of action where vitamin K is reduced. Subjects who have the more sensitive genotype usually require a lower dose than the average patient. This can be referred to the AA genotype. Those who are resistant to the effect of Coumadin usually carry the GG genotype and require stronger dosing. Conversely, the CYP2C19 gene is responsible for metabolizing active Coumadin.[14] Subjects who possess CYP2C19 variations metabolize Coumadin more slowly than subjects who don't. These patients take longer for the INR to reach a TTR and may require a lower dose of Coumadin than someone who metabolizes warfarin faster.

An article in the May, 2012 CAP Today discusses how testing for Warfarin® pharmacogenomics (PGX) is still a waiting game. No professional groups have endorsed genetic testing as a standard assay to enable Coumadin dosing. Cost is upwards of $500.00 in most reference labs. There are only two vendors who currently provide materials and a platform for Coumadin PGX analysis. In August of 2009, the Centers for Medicare and Medicaid Services decided not to reimburse testing as part of routine patient care. Nevertheless, the FDA again the following year recommended genotyping prior to initial dosing. Dr. Charles Eby at Washington University in St. Louis, states "For many, many, many physicians, it's a novelty that is not part of their practice tradition." Dr. Eby saw the FDA's statement as a positive step because the agency said it would pay for the testing if it was part of a clinical trial. In the real world, the turn-around-time of PGX testing can be as long as 30 days. Few physicians can wait that long to treat a subject with Coumadin when they need to be

anticoagulated. The controversy continues on the use of PGX for anticoagulating with OAT. For more information about Coumadin monitoring and dosing recommendation algorithms go to www.WarfarinDosing.org. The site's algorithm has been validated and used on more than 1000 patients.[17]

The Chromogenic Factor X Assay Monitors Coumadin

We have discussed how the INR is the principle method for monitoring patients on OAT. However there are many preanalytical variables that can affect the INR, for instance, the presence of a lupus anticoagulant (LA). This is especially true when the reagent and instrument combination of the test system has not been locally calibrated for the ISI. An alternative to the INR in the case of interference is the chromogenic factor X (CFX) assay. The CFX has been shown to be insensitive to many of the variables such as LA that may affect the INR.[18-22]

A number of researchers have established the CFX therapeutic range in the presence of an LA that corresponds to the therapeutic range of the INR.[23-24] Further, McGlasson (2008) postulated that the INR might be invalid due to the range "flattening out" when the INR result is >3.0. This data showed it was possible to have a subject with a very high critical value of INR and still have a CFX level in the therapeutic range.[23] This article suggests that the INR be replaced by the CFX in monitoring all subjects on Coumadin, especially for individuals with INR values >3.0 (Figure 1).

Rosborough et al showed how FII and FX activity levels did not always agree in Coumadin-treated LA subjects.[25] Their data demonstrated that the CFX method was preferred to the INR and clottable based factor assay in monitoring subjects with an LA who were on OAT. They also established that the relationship of CFX to INR testing differs during Coumadin initiation compared to during chronic Coumadin therapy.[26] Their conclusion was that this would have implications for warfarin dosage protocols in patients requiring CFX monitoring. Rosborough also described the relationship between the INR and CFX in the samples from patients in their study compared to the reciprocal transformation of the INR seen by the McGlasson study. They noted the problem with the INR when the value was >3.0 (see Figure 2).[25]

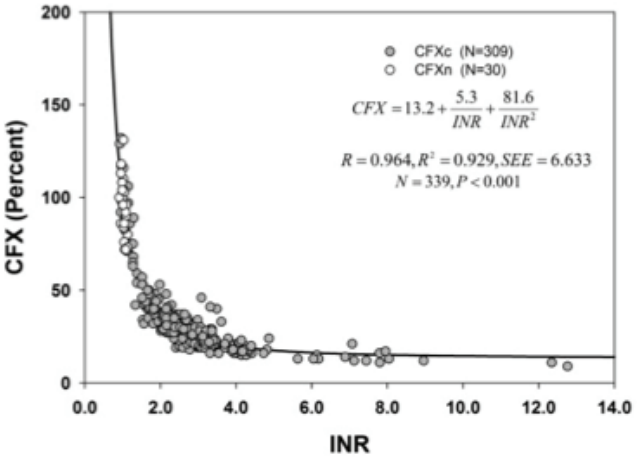

Figure 1. McGlasson DL, Romick BG Rubal BJ. Comparison of a chromogenic factor X assay with international normalized ratio for monitoring oral anticoagulation therapy. Blood Coagul Fibrinolysis 2008;19:513-7. Published with permission.

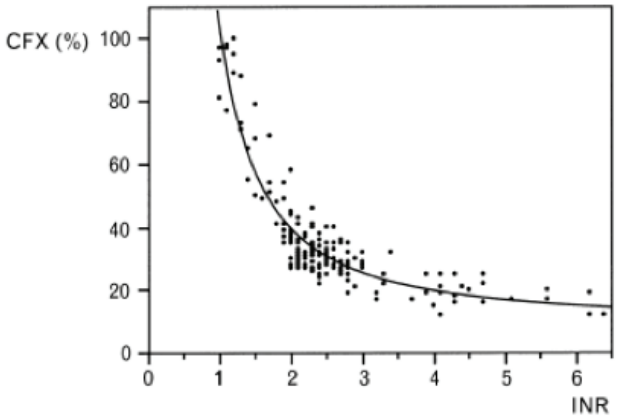

Figure 2. Rosborough TK, Jacobsen JM, Shepherd M. Factor X and factor II activity levels do not always agree in warfarin-treated lupus anticoagulant patients. Blood Coagul and Fibrinolysis 2010;21:242-4. Published with permission.

When monitoring subjects on OAT there are many pre-analytical variables that can affect the outcome of INR testing. Frequent testing in conjunction with carefully monitored anticoagulation clinics may give the patient a better chance of staying within the therapeutic range, thus enhancing their opportunity to remain incident free.

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Monitoring the Anti-Xa Anticoagulants, from Heparin to Eliquis

GEORGE A. FRITSMA

LEARNING OBJECTIVES

1. Describe the physiologic action of heparin, low molecular weight heparin, and pentasaccharide on antithrombin and activated coagulation factor X.
2. Prepare an ex vivo "Brill-Edwards curve" and employs the partial thromboplastin time to monitor unfractionated heparin.
3. Employ the prothrombinase-induced clotting time and the chromogenic anti-Xa assay to monitor unfractionated heparin, low molecular weight heparin, pentasaccharide, or direct anti-Xa therapy.

ABBREVIATIONS: AMI - acute myocardial infraction; APTT or PTT - activated partial thromboplastin time; AT - antithrombin; CAD - coronary artery disease; DTI - direct thrombin inhibitor; DVT - deep venous thrombosis; FDA - US Food and Drug Administration; GFR - glomerular filtration rate; HIT - heparin-induced thrombocytopenia with thrombosis; LMWH - low molecular weight heparin; PE - pulmonary embolism; PiCT - prothrombinase-induced clotting time; PT - prothrombin time; RI - reference interval; RUO - research use only; SERPIN - serine protease inhibitor; TAT - thrombin-antithrombin; UFH - unfractionated heparin; VTE - venous thromboembolism.

INDEX TERMS: Anticoagulants, heparin, fondaparinux, rivaroxaban, apixaban, activated partial thromboplastin time, anti-Xa heparin assay, thrombosis, thromboembolic disease, coronary artery disease.

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Standard Unfractionated Heparin Therapy

Unfractionated heparin (UFH) is a crude mixture of sulfated linear *glycosaminoglycans* extracted from porcine mucosa composed of an average of 60 saccharide units.[1] UFH is one of nature's most negatively charged molecules with a molecular weight of 3000–30,000 Daltons and a median of 15,000 Daltons.[2] A pentasaccharide sequence that binds a specific site on plasma antithrombin (AT, antithrombin III, AT III) appears on one-third of UFH molecules. This sequence provides the catalytic anticoagulant action of UFH as it binds its AT receptor site. UFH-bound AT undergoes a conformational change, exposing a second site that covalently inactivates the coagulation pathway serine proteases IIa (thrombin), IXa, Xa, XIa, and XIIa. We take clinical interest only in AT binding of thrombin and Xa, despite its additional properties. Activated AT is a *serine protease inhibitor* (SERPIN), and the protease binding reaction yields an inactive plasma complex thrombin-antithrombin (TAT).

Heparin supports the TAT "approximation" reaction. When the UFH molecule exceeds 17 linear saccharide units, thrombin assembles on the molecule in approximation to (near) the activated AT. Approximation drives the TAT reaction at four times the rate of the AT-factor Xa reaction because Xa is inactivated only by antithrombin's protease binding site, independent of approximation.

UFH lots are unrefined and vary in MW, molecule length, and anticoagulant efficacy. Individual patient UFH metabolism rates diverge markedly because human plasma and cellular proteins bind heparin at varying rates and concentrations. Consequently, laboratory monitoring is essential to UFH therapy.

Physicians administer UFH intravenously to treat deep

vein thrombosis, pulmonary embolism, and in the initial treatment of acute myocardial infarction (AMI); to prevent reocclusion after stent placement; and to maintain vascular patency during cardiopulmonary bypass graft surgery with extracorporeal circulation. Therapy begins with a bolus of 60–80 units/kg to a maximum of 5000 units, followed by continuous infusion at 12–18 units/kg/hour (Table 1). Physicians discontinue UFH at 5 days to avoid heparin-induced thrombocytopenia with thrombosis (HIT), a severe, often fatal complication in which platelets are activated by an IgG antibody that binds the heparin-platelet factor 4 complex and activates platelets.

Table 1. Unfractionated heparin therapeutic sequence

Step	Action
PTT and platelet count prior to UFH therapy	Collect lavender-closure (EDTA) and blue-closure (citrated) blood, specimen perform "baseline" PTT and platelet count
At start of UFH therapy	Bolus of 60–80 units/kg to a maximum of 5000 units
Continue with UFH therapy	Continue with 12–18 units/kg/hour for duration of therapy
Second PTT and platelet count	Collect blue- and lavender-closure specimen 4–6 hours after completion of bolus, not more than 24 hours from start of therapy, perform PTT and platelet count
Adjust dosage	Adjust dosage (drip rate) to achieve laboratory-published PTT target therapeutic range, confirm with a second PTT within 6 hours of adjustment
Subsequent PTTs and platelet counts	Repeat PTT and platelet count every 24 hours throughout duration of therapy and adjust dosage. If the platelet count drops by more than 40%, even if it remains within the reference interval, suspect HIT and discontinue immediately
Discontinue UFH at 5 days	UFH for more than 5 days increases the risk of HIT

Monitoring Unfractionated Heparin Therapy

Clinicians monitor UFH therapy closely using the partial thromboplastin time test (PTT) and platelet counts to avoid hemorrhage, rethrombosis, or heparin-induced thrombocytopenia with thrombosis (HIT). The phlebotomist collects a "baseline" blood specimen at the time the IV is started, carefully avoiding hemolysis. The specimen is assayed to ensure the non-heparin PTT is within the reference interval (RI); a prolonged baseline PTT may indicate the presence of a preexisting lupus anticoagulant, specific coagulation factor inhibitor, or factor deficiency, and confounds the therapeutic interpretation of the PTT result. In this instance, the laboratory scientist upgrades to the more reliable chromogenic anti-factor Xa heparin assay. A baseline platelet count is necessary to compare with later platelet counts, as a drop of more than 40%, even if the platelet count remains within the RI, signals the risk of HIT.

The phlebotomist collects a second specimen 4–6 hours after completion of the bolus administration, and not more than 24 hours from initiation of therapy and another PTT is performed. The result of the second specimen should fall within the therapeutic range, which is established in the laboratory (see the next section) and which is published with the result. The clinician adjusts the infusion rate to ensure the PTT result remains within the target range, repeating the PTT every 6 hours. Once the dosage is stable, the PTT is subsequently repeated every 24 hours until anticoagulation is discontinued. The laboratory scientist also monitors the platelet count daily. A 40% or greater reduction in platelet count, even if the count remains within the reference interval, is evidence for HIT. If HIT is suspected, UFH is immediately discontinued and replaced with pentasaccharide (fondaparinux) therapy or a direct thrombin inhibitor (DTI) such as argatroban.[3]

Determining the Partial Thromboplastin Time Therapeutic Range

Hemostasis laboratory scientists establish and communicate a PTT therapeutic range for UFH therapy. The scientist collects 50 or more specimens from patients receiving UFH at all levels of anticoagulation and performs PTTs on all.[4] The specimens must be from patients who are not receiving Coumadin therapy; their PT results must be within the PT RI. Chromogenic anti-factor Xa heparin assays are performed on all specimens, and the paired PTT and anti-Xa results are displayed in a linear graph. The range

in seconds of PTT results that corresponds to 0.3–0.7 anti-factor Xa heparin units/mL is the therapeutic range. This is known as the *ex vivo* or "Brill-Edwards" method for establishing the heparin therapeutic range of the PTT and is required by proficiency testing and accreditation agencies (Figure 1).

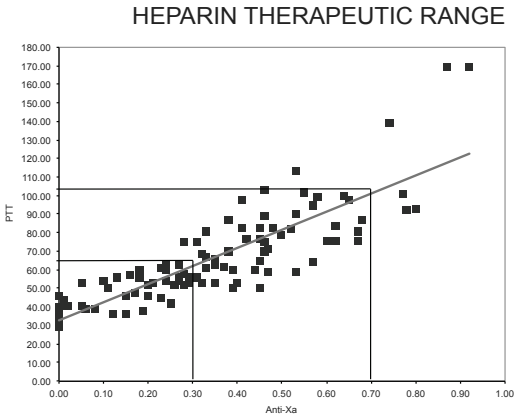

Figure 1. Ex vivo "Brill-Edwards" curve to determine the UFH therapeutic range. PTTs and chromogenic anti-Xa assays are performed on at least 50 UFH specimens with normal PTs and on 20 normal subjects. Results are expressed as an XY graph. The PTT range in seconds that corresponds to 0.3–0.7 heparin anti-Xa units/mL is the target therapeutic range. A new Brill-Edwards curve is plotted with each change in PTT reagent lot.

The scientist reports the PTT result, the RI, and the current UFH therapeutic range. Because reagent sensitivity varies among producers and among individual producers' reagent lots, the clinician must evaluate PTT results in relationship to the institution's therapeutic range and RI, which may vary with each lot change. The PTT is typically used to measure the effects of UFH, however the chromogenic anti-factor Xa heparin assay may be used to assay UFH, low molecular weight (LMWH), and pentasaccharide (fondaparinux).[5]

Limitations of the Partial Thromboplastin Time

Several interferences reduce PTT sensitivity, a circumstance called *heparin resistance*. (Table 2) Inflammation may be accompanied by hyperfibrinogenemia exceeding 400 mg/dL and von Willebrand factor or coagulation factor VIII activities over 150%. Both reduce the PTT's response to heparin. Further, AT may become depleted in prolonged therapy or when there is an inherited or acquired underlying AT deficiency. The PTT result remains within the reference interval or is only slightly prolonged despite increasing heparin dosages.

Table 2. Limitations of the PTT

"Heparin resistance;" PTT is insensitive to UFH therapy
- Hyperfibrinogenemia: fibrinogen exceeds 400 mg/dL in acute inflammation
- Coagulation factor VIII or von Willebrand factor over 150%
- AT becomes depleted during UFH therapy, congenital or acquired AT deficiency

Prolonged baseline PTT
- Hypofibrinogenemia: fibrinogen level below 100 mg/dL
- Congenital or acquired coagulopathy: single or multiple factor deficiency
- Specific factor inhibitor, most often factor VIII inhibitor
- Lupus anticoagulant: non-specific inhibitor
- Circulating fibrin degradation products or paraproteins

Hypofibrinogenemia, factor deficiencies, specific factor inhibitors, lupus anticoagulant, and the presence of fibrin degradation products or paraproteins prolong the PTT independent of heparin levels, rendering the assay overly sensitive and inaccurate for UFH monitoring.[6] In instances of resistance or prolonged baseline PTT, the laboratory scientist upgrades to the chromogenic anti-factor Xa heparin assay.

Platelets in anticoagulated blood specimens release platelet factor 4 (PF4), a heparin-neutralizing protein. In specimens from patients on UFH therapy, the PTT begins to shorten one hour after collection because of *in vitro* PF4 release. The specimen must be centrifuged to produce platelet-poor plasma with a platelet count below 10,000/μL, and the plasma must be removed from the cells. The PF4 interferes with both the PTT and the chromogenic anti-Xa heparin assay.[7]

The Activated Clotting Time

The activated clotting time (ACT), a point-of-care assay, is used to monitor high-dose heparin therapy in the cardiology surgical suite.[8] ACT assay and instrument distributors provide evacuated specimen collection tubes that contain kaolin, a particulate clot activator. The fresh whole blood specimen is placed in the reaction well of, for instance, the Hemochron® Signature Elite (International Technidyne, Inc, Piscataway, NJ), where it is rotated and continuously monitored. When a clot forms, a magnet positioned within the sample is pulled away from a sensing device, stopping the timer. The RI of the ACT is typically 90–

175 seconds. The ACT is particularly useful for monitoring the high blood levels (1–2 units/mL) of UFH employed during coronary artery bypass surgery, which prolong the result to 200–400 seconds. Laboratory scientists seldom perform the ACT within the laboratory, but are called upon to assist with validation and troubleshooting of the operating room-based instrument.

The Prothrombinase-induced Clotting Test

The prothrombinase-induced clotting test (PiCT®, Centerchem, Inc, Norwalk, CT; Pentapharm, Basel, Switzerland) employs a reagent composed of activated factor X (Xa), phospholipid, and Russell viper venom-V, a venom component that activates coagulation factor V.[9] The PiCT assay may be used to monitor UFH, LMWH, pentasaccharide, the oral direct anti-Xa anticoagulants rivaroxaban and apixaban, and the DTIs.

In the case of Heparin, LMWH and pentasacharide, the scientist adds patient plasma to the reagent and incubates 3 minutes, during which time UFH-AT, LMWH-AT, and pentasaccharide-AT complexes are formed. In the case of direct anti-Xa drugs, the incubation time is omitted to avoid an AT effect that leads to an initial lowering of the clotting time at low concentrations of direct anti-Xa drug. This step results in an inhibition of a proportion of the FXa present in the reagent. The scientist next adds $CaCl_2$ and starts a timer. The residual Xa complexes with phospholipid and with the Va generated from the plasma by the RVV-V. The interval to clot formation is recorded and compared to a standard curve. The PiCT assay is under ongoing clinical studies to obtain FDA clearance as an aid in the monitoring of UFH therapy. Future studies will follow regarding all of the other drugs listed above. This test may prove to be the most versatile of the clot-based anticoagulant assays.

Reversal of Unfractionated Heparin Using Protamine Sulfate

Protamine sulfate, a positively charged protein extracted from salmon sperm, neutralizes UFH at a ratio of 100 units of heparin per mg of protamine sulfate.[10] The physician administers protamine sulfate by slow intravenous push. The effect is detected by the instant shortening of the PTT or ACT. Protamine sulfate also neutralizes LMWH, although the neutralization is incompletely reflected in the results of the anti-factor Xa heparin assay. Paradoxically, an overdose of protamine sulfate may cause hemorrhage.

Low Molecular Weight Heparin

LMWH: Depolymerized Unfractionated Heparin

Uncertainty about UFH dose response led to the development of LMWH, which was cleared for prophylaxis in 1993. In the US, enoxaparin (Lovenox®, Aventis Corp) commands the major market share of LMWH preparations. LMWH is prepared from UFH using chemical or enzymatic fractionation. Fractionation yields a median molecular weight of 4500–5000 Daltons, about one third the mass of UFH. LMWH possesses the same active pentasaccharide sequence as UFH; however, the overall shorter polysaccharide chains provide somewhat less space for thrombin approximation, so the AT to thrombin neutralization response is reduced. The AT to factor Xa neutralization response is unchanged, however, as the Xa reaction does not rely on approximation, so LMWH provides nearly the same anticoagulant efficacy as UFH, predominantly through Xa inhibition.

Patients self-administer LMWH by subcutaneous injection once or twice a day using premeasured syringes at selected dosages, for instance, 30 mg every 12 hours or 40 mg once daily. Prophylactic applications provide coverage during or after neurosurgery and orthopedic surgery and after trauma, typically for 14 days from the time of the event. Hematologists also use LMWH to treat DVT, PE, and unstable angina. When Coumadin patients require surgery, Coumadin is discontinued for up to a week before the procedure and replaced with LMWH, which has a shorter half-life, produces less risk of bleeding, and may be partially reversed with protamine sulfate.

LMWH's advantages are rapid bioavailability after subcutaneous injection, making IV administration unnecessary; half-life of 3–5 hours compared with 1–2 hours for UFH; and a fixed dose response that reduces the need for laboratory monitoring. The risk of HIT is reduced by 90% in people who have never received heparin before, however LMWH reacts with previously formed HIT antibodies. The risk of LMWH-induced bleeding is equivalent to UFH, about 10%.

Laboratory Assay of Low-Molecular-Weight Heparin

LMWH is cleared by the kidneys alone, so it

accumulates in renal insufficiency. A laboratory assay is necessary when the glomerular filtration rate (GFR) is less than 30 mL/min or the serum creatinine exceeds 4 mg/dL (Table 3). Creatinine assays are run periodically to document kidney function and avoid the risk of LMWH accumulation.

To monitor LMWH, the phlebotomist collects a specimen 4 hours after subcutaneous injection and the platelet-poor plasma is tested using the anti-factor Xa heparin assay, which employs a fixed concentration of factor Xa and a chromogenic substrate specific to the enzymatic properties of Xa. LMWH forms a complex with AT that may be supplied by the reagent or may be provided by the patient plasma. The LMWH-AT complex inactivates reagent factor Xa. A measured excess of factor Xa digests the substrate, yielding a colored product whose intensity is inversely proportional to the initial heparin concentration.

Table 3. Reasons for performing a laboratory assay of low molecular weight heparin

It is not necessary to monitor LMWH therapy routinely, however it must be assayed when there is a fluid imbalance or when the coagulation system is unstable:

- Renal disease: glomerular filtration rate less than 30 mL/m or serum creatinine exceeds 4 mg/dL
- Abnormal blood and cellular fluid distribution in morbid obesity or in the excessively slender
- People over 70 whose population was not included in clinical trials
- Children
- Pregnancy
- Cancer
- Diabetes
- Chronic inflammation
- Liver disease

To prepare a standard curve, the laboratory scientist obtains a characteristic lot of LMWH from the pharmacy and prepares dilutions that correspond with the intended therapeutic range. If the chromogenic anti-factor Xa heparin assay is to be used to monitor UFH and LMWH, a single hybrid standard curve may be prepared.[11] A separate curve may be necessary to monitor pentasaccharide (fondaparinux). The therapeutic range for twice-daily LMWH regimens is 0.5–1 units/mL and for once-daily regimens is 1–2 units/mL.

The anti-factor Xa heparin assay and the PiCT are the only assays available to monitor LMWH and pentasaccharide therapy. They may also be used in place of the PTT to assay UFH with little or no modification and may substitute for the PTT when clinical or laboratory conditions render the PTT unreliable. The chromogenic anti-factor Xa heparin assay is also the reference method for establishing the PTT therapeutic range in the Brill-Edwards curve procedure, discussed previously.

Pentasaccharide (Fondaparinux) Therapy

Fondaparinux sodium (Arixtra®; GlaxoSmithKline, Research Triangle Park, NC) is a synthetic formulation of the active pentasaccharide sequence in UFH and LMWH. Fondaparinux is equivalent in clinical efficacy and safety to UFH and LMWH, has a reproducible dose response and a half-life of 12–17 hours, requiring once-a-day subcutaneous injections of 2.5 mg each. Fondaparinux is approved for surgical prophylaxis and for the treatment of DVT and PE but is contraindicated for patients with a GFR less than 30 mL/min or with body weights less than 50 kg.[12]

Scientists employ the PiCT or the chromogenic anti-factor Xa heparin assay to monitor fondaparinux therapy when necessary. Blood is collected four hours after injection, and the target range is 0.14–0.19 mg/L. The laboratory scientist prepares a standard curve using fondaparinux, not UFH, LMWH, nor a hybrid standard, because concentrations are expressed in mg/L and not units/dL.

Rivaroxaban and Apixaban, Oral Direct Xa Inhibitors

Oral rivaroxaban (Xarelto®; Bayer Healthcare AG, Leverkusen, Germany; Ortho-McNeil Pharmaceuticals, Inc, Raritan, NJ) and oral apixaban (Eliquis®, Bristol-Myers Squibb and Pfizer, New York, NY) function unmodified to inhibit Xa, bypassing the need for AT. Both have favorable efficacy and safety outcomes when compared to UFH, LMWH or Coumadin.[13] Both appear to provide attractive alternatives to Coumadin and LMWH injections because they are convenient and require little laboratory monitoring. Rivaroxaban was FDA-cleared July 1, 2011 for VTE prophylaxis in patients who are undergoing total knee replacement or total hip replacement surgery. Apixaban was cleared in December, 2012.[14] The standard oral rivaroxaban dosage is 10 mg/day and because of its predictable pharmacody-namics, laboratory monitoring is seldom

necessary. Rivaroxaban and apixaban prolong the PT and PTT, as reported in several clinical trials. PTT responses vary widely among reagents and reagent lots, thus no attempt has been made to monitor either anticoagulant with the PTT. The PT, however, provides a reproducible linear relationship with rivaroxaban.[15] Rivaroxaban (and by generalization, apixaban) may also be assayed using the chromogenic anti-Xa heparin assay by standardizing with rivaroxaban (or apixaban) in place of UFH, LMWH or fondaparinux, though it remains for laboratory scientists to correlate laboratory results with clinical outcomes. In September, 2012, Diag-nostica Stago, Inc announced the availability of rivaroxaban control and calibrator plasmas to be used with their chromogenic anti-Xa kit.

The pharmaceutical industry and the in vitro diagnostics industry have together made significant strides, beginning with crude UFH that is typically administered intravenously, LMWH and pentasaccharide in predictable doses administered subcutaneously, and now rivaroxaban and apixaban, both safe and effective oral preparations. We've made modest progress towards drugs that are both effective and safe, threatening to topple Coumadin and heparin from their lofty perches, however there are three wrinkles left to iron out. No laboratory assay has been FDA-cleared for monitoring rivaroxaban or apixaban. All current kits, controls, and calibrators are labeled for research use only. While safe and effective, the anticoagulant properties of rivaroxaban and apixaban have both rapid onset and rapid regression. Consequently, compliance must be 100%; one missed dosage places the patient in danger of rethrombosis. And, unlike UFH and LMWH, no effective reversal agent has been developed for fondaparinux, rivaroxaban, or apixaban, thus leaving the emergency department with only uncertain solutions for patients at risk for an intracranial hemorrhage. These issues are soon to be resolved, revolutionizing the field of anticoagulation.

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Monitoring the Direct Thrombin Inhibitors

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LEARNING OBJECTIVES

1. Diagram the physiology of the direct thrombin inhibitors
2. Distinguishe between argatroban and bivalirudin.
3. Provide the means for monitoring direct thrombin inhibitors.

ABBREVIATIONS: APTT or PTT - activated partial thromboplastin time; AT - antithrombin; CAD – coronary artery disease; DTI - direct thrombin inhibitor; DVT - deep venous thrombosis; ECT-ecarin clotting time; FDA - US Food and Drug Administration; HIT - heparin-induced thrombocytopenia with thrombosis; INR - international normalized ratio; LMWH - low molecular weight heparin; PE - pulmonary embolism; PCI - percutaneous intervention (cardiac catheterization); PiCT - prothrombinase-induced clotting time; PT - prothrombin time; RI - reference interval; RUO - research use only; TCT - thrombin clotting time; TPA - tissue plasminogen activator; UFH - unfractionated heparin; VTE - venous thromboembolism.

INDEX TERMS: Anticoagulants, heparin, Coumadin, lepirudin, argatroban, bivalirudin, dabigatran, prothrombin time, activated partial thromboplastin time, atrial fibrillation, thrombosis, thromboembolic disease, coronary artery disease, heparin-induced thrombocytopenia with thrombosis, thrombin clotting time, dilute thrombin time, ecarin clotting time, prothrombinase-induced clotting time.

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The direct thrombin inhibitors (DTIs), intravenous argatroban and bivalirudin and oral dabigatran, reversibly bind and inactivate free and clot-bound thrombin without activating antithrombin (AT, antithrombin III, ATIII), thereby suppressing coagulation at the final stage of the cascade.[1] Argatroban or bivalirudin are substituted for unfractionated heparin (UFH) or low molecular weight heparin (LMWH) when heparin-induced thrombocytopenia with thrombosis (HIT) is suspected or confirmed, as they do not interact with the HIT antibody.[2] When HIT is suspected, the risk of either venous or arterial thrombosis is 50% for 30 days after heparin withdrawal unless argatroban or bivalirudin is employed. Coumadin is contraindicated in HIT as it is prothrombotic for the first 5 days of administration. Dabigatran has been cleared for prevention of venous thromboembolic (VTE) disease in Europe and Canada and for prevention of stroke in atrial fibrillation in the US. As the first new oral anticoagulant cleared by the FDA since 1954, dabigatran is beginning to take its place in the formulary. Laboratory scientists are challenged to find clinically effective means to monitor its plasma concentration.

Argatroban

Argatroban (Novostan®; GlaxoSmithKline, Research Triangle Park, NC) is a non-protein l-arginine derivative with a molecular weight of 527 Daltons.[3] Argatroban was FDA-cleared in 1997 for anticoagulation in HIT.[4] Argatroban may be used for prophylaxis, treatment, and also for anticoagulation during percutaneous cardiac intervention (PCI, cardiac catheterization) for patients with HIT.[5]

The physician initiates the argatroban intravenous infusion at 2 μg/kg/min or in patients with liver disease at 0.5 μg/kg/min. During PCI, a bolus of 350 μg/kg is administered over 3–5 minutes followed by 25 μg/kg/min. Argatroban is cleared by the liver and excreted in stool. There is a 5% general bleeding risk and no direct antidote; however, the half-life is 51

minutes, and argatroban clears from the blood completely in 2–4 hours except in liver disease. Argatroban therapy may require laboratory testing, particularly for detection of an overdose or in liver disease.

Lepirudin, a Synthetic Analogue of Hirudin

Lepirudin (Refludan®; ZLB Behring GmbH, Montville, N.J., and Marburg, Germany) is a recombinant 7000 Dalton protein DTI. Lepirudin is an analogue of natural *hirudin*, which is produced in trace amounts by the medicinal leech *Hirudo medicinalis.* Hirudin is secreted in leech saliva, enabling the leech to avoid clotting while ingesting its blood meal. Lepirudin is approved for intravenous anticoagulation in HIT. It binds free but not fibrin-bound thrombin, and is cleared only by the kidneys.

Lepirudin production was discontinued May 31, 2012, though existing stocks may be used until depleted. Laboratory assays may be necessary because the risk of overdose-related bleeding is 10%. The dosage must be reduced in the presence of elevated serum creatinine or reduced glomerular filtration rate (GFR) and is discontinued when the GFR is less than 15 mL/min or the creatinine exceeds 6 mg/dL. Serial measurements of GFR or serum creatinine are necessary throughout lepirudin therapy. There is no antidote for lepirudin overdose, but the plasma half-life is only 20 minutes, provided kidney function is normal.

Anti-hirudin antibodies form in about 40% of HIT patients who are treated with lepirudin.[6] These antibodies complex with lepirudin and may actually increase lepirudin's anticoagulant effect, because the bound DTI remains active and the complex clears slowly. Strict laboratory monitoring is necessary during prolonged therapy, and anaphylaxis may occur during a second administration, thus lepirudin may only be used once.[7]

Bivalirudin, a Depolymerized Lepirudin Derivative

Bivalirudin (Angiomax®; The Medicines Company, Parsippany, NJ) is a synthetic 20-amino acid peptide derivative of lepirudin with a molecular weight of 2180 Daltons. Bivalirudin inactivates both free and clot-bound thrombin. Bivalirudin was FDA-cleared in 2000 for use as an anticoagulant in patients with unstable angina at risk for HIT who are undergoing PCI.[8] Bivalirudin is intended for use with concurrent aspirin therapy at a dosage of 325 mg/day and has been studied only in patients receiving aspirin.[9] In the cardiac catheterization laboratory, physicians provide an intravenous bolus dose of 0.75 mg/kg followed by an infusion of 1.75 mg/kg/hr for the duration of the PCI. After 4 hours, an additional intravenous infusion may be given at a rate of 0.2 mg/kg/hr for up to 20 hours.

The rate of major hemorrhage associated with bivalirudin is 4%. There is no way to neutralize bivalirudin; however, in patients with normal renal function the half-life is 25 minutes. The dosage is decreased in patients with reduced GFR or elevated serum creatinine. Because bivalirudin is a small peptide, it does not elicit an immune response, thus no anti-hirudin-like antibodies to bivalirudin have been described.

Dabigatran, the Oral Direct Thrombin Inhibitor

Dabigatran etexilate (Pradaxa®; Boehringer Ingelheim, Ingelheim, Germany) is an oral pro-drug that converts upon digestion to active dabigatran, a DTI that binds both free- and clot-bound thrombin.[10] Dabigatran's efficacy and safety appear to match those of LMWH and Coumadin, and it has no interaction with food.[11] It is cleared by the kidneys, has a half-life of 12–17 hours, and is not metabolized by liver cytochrome enzymes. Dabigatran causes no liver toxicity. In the spring of 2009, dabigatran was cleared by agencies in Canada and northern Europe at 150 mg once a day for venous thromboembolic (VTE) disease prophylaxis following total knee replacement or total hip replacement surgery.[12] The US FDA cleared dabigatran for prevention of ischemic stroke in patients with atrial fibrillation in the fall of 2010, making dabigatran the first oral anticoagulant to be cleared since Coumadin in 1954.

Dabigatran caused dyspepsia in 11% of clinical trial subjects. Its anticoagulant effect is immediate, but a single missed dose may expose the patient to renewed thrombosis risk. In case of overdose associated with bleeding, renal dialysis is the only means for reducing plasma levels, as there is no current medical means for neutralizing its effect. The question of laboratory monitoring in pregnant patients, the elderly, morbidly obese or underweight adults, and patients with renal dysfunction was not addressed during dabigatran

clinical trials, but has become an initiative since the drug was released.

Measuring Direct Thrombin Inhibitor Therapy

All three DTIs prolong the PT, PTT, thrombin clotting time (TCT), and the activated clotting time (ACT). For nonsurgical therapy, argatroban and bivalirudin may be assayed using the PTT. The target therapeutic range is 1.5–3.0 times the mean of the laboratory-established reference interval (RI). Blood is collected 4 hours after the initiation of intravenous therapy for bivalirudin or 2 hours after starting argatroban, and the dosage is adjusted to achieve a PTT in the therapeutic range. Though generally available, the PTT is not specifically cleared for DTI monitoring, and there exist considerable sensitivity variations among reagent formulations and lots. However, because the duration of argatroban and bivalirudin therapy is relatively short, no attempt is being made to standardize the PTT for DTI monitoring.

The ACT may be used to assess argatroban or bivalirudin during PCI or coronary artery bypass graft surgery.[13] During these procedures, the target ACT is 320–400 seconds (RI 90–175 seconds).

PTT reagents are not calibrated specifically to monitor argatroban or bivalirudin. Further, in instances in which the baseline PTT is prolonged by lupus anticoagulant, specific inhibitors, or factor deficiencies, the ecarin clotting time (ECT) is an attractive alternative for monitoring DTI therapy, including dabigatran therapy. Ecarin (ecarinase; Pentapharm, Basel, Switzerland) is an enzyme extracted from *Echis carinatus* venom that converts prothrombin to the intermediate *meizothrombin,* which converts fibrinogen to fibrin.[14] DTIs bind meizothrombin and generate a linear, dose-dependent prolongation of the ECT. Aside from DTIs, the ECT is prolonged only by abnormally low prothrombin and fibrinogen activity. Diagnostica Stago Inc has developed both a clot-based and a chromogenic ecarin assay, however both await FDA clearance.

Dabigatran, though it requires no regular monitoring, represents a special case compared to argatroban and bivalirudin. Its half-life of 12–17 hours and its indication for long-term therapy make it more frequently implicated in hemorrhage or thrombosis.[15] Consequently, dabigatran should be assayed in all the same circumstances as LMWH, as listed in *Monitoring the Anti-Xa Anticoagulants*. Further, it may be necessary to check for dabigatran compliance or to identify dabigatran in emergent hemorrhage.

Attempts to assay dabigatran using traditional coagulation tests have revealed that the PT is non-linear at therapeutic levels and that PTT reagents vary widely in dabigatran sensitivity.[16] The TCT is linear but too sensitive. The TCT may be used semiquantitatively; a normal TCT indicates dabigatran absence, a TCT that exceeds 100 seconds may imply an overdose or renal retention. Though rarely used, the reptilase time assay is insensitive to dabigatran, thus a prolonged and uncorrectable PT, PTT, and TCT associated with a normal reptilase time result may indicate dabigatran.

Dabigatran may be assayed using the ECT or the PiCT assay described in the accompanying article, *Monitoring the Anti-Xa Anticoagulants*. BIOPHEN's Hemoclot® thrombin inhibitor assay, also known as the dilute TCT, is a simple procedure in which test plasma is mixed with normal plasma and the mixture is assayed using TCT reagent. This assay is linear and stable. Finally, the Helena point of care Abrazo® DTM provides near-patient testing based on the ECT. Regrettably, all current and proposed assays for dabigatran, and for the new oral anti-Xa anticoagulants rivaroxaban and apixaban are currently stalled at the FDA awaiting clearance. Meanwhile, laboratory operators are making do with the PTT, anti-Xa and the TCT. Given the efficacy, safety, and convenience of the new oral anticoagulants, these drugs and their assays may soon be made available worldwide.

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Continuing Education Questions

WINTER 2013

1. What was the first anticoagulant to be released by the US Food and Drug Administration?
   a. Hirudin
   b. Heparin
   c. Coumadin
   d. Argatroban

2. What anticoagulant functions by raising plasma antithrombin activity?
   a. Hirudin
   b. Heparin
   c. Coumadin
   d. Argatroban

3. What anticoagulant functions by reducing the activity of vitamin K epoxide reductase?
   a. Hirudin
   b. Heparin
   c. Coumadin
   d. Argatroban

4. What laboratory assay is used to find the plasma concentration of low molecular weight heparin (LMWH)?
   a. Prothrombin time with international normalized ratio (PT/INR)
   b. Activated partial thromboplastin time (APTT, PTT)
   c. Chromogenic anti-Xa heparin assay
   d. Thrombin time

5. What anticoagulant is an analogue of an anticoagulant protein in the saliva of the medicinal leech?
   a. Hirudin
   b. Heparin
   c. Coumadin
   d. Argatroban

6. What is the most common method for monitoring unfractionated heparin (UFH)?
   a. Prothrombinase-induced clotting time (PiCT)
   b. Chromogenic anti-Xa heparin assay
   c. PT/INR
   d. PTT

7. What method may be used to monitor UFH, LMWH, direct anti-Xa inhibitors, and direct thrombin inhibitors (DTIs)?
   a. Chromogenic anti-Xa heparin assay
   b. PT/INR
   c. PiCT
   d. PTT

8. What is the generic name of the synthetic anticoagulant that mimics the active pentasaccharide sequence of UFH and LMWH?
   a. Fondaparinux
   b. Rivaroxaban
   c. Argatroban
   d. Apixaban

9. What is the generic name of the first oral direct anti-Xa anticoagulant to be FDA-cleared?
   a. Fondaparinux
   b. Rivaroxaban
   c. Argatroban
   d. Apixaban

10. Name two laboratory assays that may be used to monitor the new oral direct anti-Xa anticoagulants.
    a. PT and PTT
    b. PT and anti-Xa heparin assay
    c. PTT and anti-Xa heparin assay
    d. Thrombin time and anti-Xa heparin assay

11. Which of the following expresses the sensitivity of a PT reagent?
    a. International reference preparation (IRP)
    b. International sensitivity index (ISI)
    c. World health organization (WHO)
    d. Chromogenic factor X (CFX)

12. Which expression is *not* required to calculate the INR?
    a. ISI
    b. Patient PT results in seconds
    c. Dilute thrombin time in seconds
    d. Geometric mean normal PT (GMNPT) in seconds

13. What is the ideal INR therapeutic range for a subject on Coumadin therapy?
    a. 1.5–2.5
    b. 2.0–3.0
    c. 3.0–4.0
    d. 3.5–4.5

14. Which of the following statements is true?
    a. The VKORC1 gene controls the rate of unfractionated heparin clearance by the kidneys.
    b. The VKORC1 gene regulates the clearance of Coumadin by moderating liver function.
    c. The VKORC1 gene controls the sensitivity of a patient's response to Coumadin.
    d. The VKORC1 gene metabolizes the active component in Coumadin.

15. Which assay should be used in place of the INR when a subject who is on Coumadin has a lupus anticoagulant (LA)?
    a. Clot-based coagulation factor X
    b. Chromogenic factor X
    c. FVIII
    d. PTT

16. When are argatroban or bivalirudin used?
    a. Any time heparin may be used
    b. They are used only during PCI
    c. Any time Coumadin may be used
    d. In place of heparin in patients with HIT

17. What test is commonly used to assay argatroban or bivalirudin?
    a. PT
    b. PTT
    c. TCT
    d. Anti-Xa

18. Which test may be used to monitor dabigatran?
    a. PT
    b. PTT
    c. TCT
    d. ECT

19. What is the only way to reduce dabigatran in overdose-related bleeding?
    a. Renal dialysis
    b. Fresh-frozen plasma
    c. Recombinant activated factor VII
    d. Activated prothrombin complex concentrate

20. What DTI is cleared by the liver?
    a. Dabigatran
    b. Argatroban
    c. Bivalirudin
    d. Lepirudin

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3.	a	b	c	d	14.	a	b	c	d
4.	a	b	c	d	15.	a	b	c	d
5.	a	b	c	d	16.	a	b	c	d
6.	a	b	c	d	17.	a	b	c	d
7.	a	b	c	d	18.	a	b	c	d
8.	a	b	c	d	19.	a	b	c	d
9.	a	b	c	d	20.	a	b	c	d
10.	a	b	c	d					
11.	a	b	c	d					

Participant Information
Please circle the most appropriate answers.

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

2. Did these articles achieve their stated objectives?

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

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

Answers to 2010 FOCUS Continuing Education Questions

23(1) Winter 2010
Obesity and Metabolic Syndrome

1. Obesity is defined as a BMI of greater than:
   c. > 30%

2. Which of the following hormones is a component of the HPA system?
   c. TSH

3. After leptin feedback indicates adequate adiposity, which of the following is not among the three primary ways that the hypothalamus helps regulate body weight?
   d. restriction of thermogenesis

4. Which of the following statements correctly describes the molecular types for leptin, insulin and cortisol?
   a. Cortisol is a steroid, the other two are peptide hormones.

5. Which of the following is least likely to be considered an "entrance ramp" onto the "Highway to Obesity"?
   a. Grave's disease

6. Leptin resistance results in each of the following, except:
   b. appetite suppression

7. Which of the following best describes the concept of "lipid buffering" as it restricts ectopic fat deposition?
   d. Non-adipocytes are stimulated to increase lipolysis and decreased re-esterification, whereas the opposite is true for adipocytes.

8. Which of the following does not involve the dopamine "pleasure/reward system"?
   d. genetics

9. Which of the following best describes inheritance on an individual patient's tendency to become obese?
   b. Inheritance of the tendency to become obese is a complex polygenic involving multiple alleles, each with a modest impact on BMI.

10. Which of the following analytes are least likely to be elevated in serum from an obese patient?
    c. adiponetin

11. Identify the two researchers usually credited with originally defining the concept of metabolic syndrome:
    b. Kaplan and Reaven

12. Which of the following best describes the origin of plasma FFAs (free or non-esterified fatty acids) and the significance of elevated plasma FFA levels?
    c. FFAs are released from adipocytes during fasting conditions and elevated FFAs tend to be toxic to many cell types.

13. Researchers (including Dr. Richard Unger) have investigated several possible physiologic roles for leptin. Indicate which role of leptin that appears to be independent of the hypothalamus.
    a. preventing ectopic fat deposition

14. Which of the following pairs is <u>not</u> listed among the criteria for diagnosis of metabolic syndrome?
    a. elevated hsCRP and high FFAs

15. Which of the following does <u>not</u> occur as a result of elevated FFAs?
    c. most FFAs are engulfed by foam cells to form plaque

16. Leptin resistance results in the loss of all of the following, except:
    d. stimlation of lipolysis

17. Leptin normally promotes lipid buffering in fat cells but inhibits re-esterification in non-adipocytes. Which of the following best describes the changes that allow ectopic fat deposition when leptin resistance occurs?
    b. Leptin no longer inhibit re-esterification in non-adipocytes

18. Ceramide is a toxic compound formed from the reaction of which of the following pairs of compounds
    c. serine and palmitate

19. Metabolically healthy obese individuals would tend to have each of the following characteristics (compared obese individuals with metabolic syndrome) except:
    d. increased sensitivity to leptin

20. Which of the following best describes how leptin binding to its receptor can activate metabolism at both transcriptional level and activation of pre-existing enzymes.
    d. The PPAR-α system controls transcription, while AMPK causes phosphorylation of existing enzymes.

21. Adiponectin (ADN) is an "adipokine" with all of the following characteristics, except:
    d. ADN levels are directly proportional to fat mass

22. ADN inhibits the development of atherosclerosis by limiting all of the following effects except:
    b. Increased oxidation of LDLs

23. Which of the following best explains why glycerogenesis and the activation of the PEPCK enzyme must occur during lipid buffering?
    a. The glycerol released during lipolysis is exported and must be replaced by new synthesis of glycerol, using the enzyme PEPCK.

23(2) Spring 2010
Nanotechnology

1. Which of the following scientists was the first to lecture about the possibility of manipulating atoms?
    c. Richard Feynman

2. The sixty carbon atoms resembling soccer balls have been termed:
    a. Fullerenes

3. An application in the military for nanotechnology is:
    a. Body armor

4. Nanotechnology is defined as technology and research in the range of:
    c. 1-100 nanometers

5. An unfolded nanotube might best be described as resembling
    b. chicken wire in hexagonal lattices.

6. Nanotechnology drug delivery is:
    a. less toxic than traditional chemotherapy

7. A nanotechnology FDA approved test for respiratory viruses uses:
    c. Gold nanoparticles

8. A nanotechnology procedure has been developed for which of the following bacteria?
    c. Listeria monocytogenes

9. Which of the following elements has been used to repair broken bones?
    d. Titanium

10. Nanotechnology may provide a means for more efficiently producing:
    a. food, energy and a healthier environment.

11. Some scientists believe that nanotechnology will:
    b. enable construction of nanomachines atom-by-atom.

12. Nanotechnologists predict that:
    d. 'a' and 'b' are both true.

13. Which predicted 21st century crises will nanotechnology attempt to overcome?
    d. accelerating industrialization, population growth, starvation, natural resource depletion and environmental deterioration

14. Regarding nanotechnology regulatory agencies, which statement is most accurate?
    c. The EPA has recently announced new research into nanomaterial safety.

15. What type of exposure is most likely to potentially put you "at risk" with nanomaterials?
    c. lungs

16. With regard to the ability of nanoparticles to penetrate skin, the data show (at present) that:
    a. many scientists disagree at present on this issue, and there are conflicting data.

17. The most widely accepted information about safety and documented information for laboratory professionals comes (originally) from:
    a. data in the peer-reviewed scientific literature.

18. In what products are nanomaterials already present?
    d. in cosmetics, food additives, sports gear, and some clinical diagnostic kits.

19. Which agency was formed by the FDA to evaluate specific problems that may arise from using nanomaterials?
    c. Nanotechnology Task Force

20. Where can I find the most reliable source of information concerning laboratory safety that is currently accepted about nanomaterial safety?
    a. Material Safety Data Sheets

23(3) Summer 2010
Educational Technology

1. A key element of competency based education and training is that it is:
   d. Structured for mastery of specific knowledge and tasks

2. An example of a course module as part of CBET medical laboratory curriculum would be:
   b. Biochemical Diagnostic Testing

3. The process of competency based education and training begins with:
   c. Performing functional analysis of occupational roles

4. The medical laboratory curriculum in developing countries needs to:
   a. Be connected with scope of practice

5. Technology that is commonly used in medical laboratory programs in developing countries include:
   b. Presentation slides to illustrate principles of lab techniques

6. Identify the true statement regarding technology uses in developing countries:
   d. Case studies and lecture notes can be shared with students on their flash drives

7. What is the primary mode of communication between the instructor and learners in an online course?
   d. Course management system

8. Social presence is important in online education because learners must:
   a. feel that they are part of a learning community

9. Which of the following strategies will motivate an online learner to actively participate in an online course?
   b. Requiring discussion board as a part of the student grade

10. One reason that teaching a online course is more time consuming than a face-to-face course is that an online course requires
    d. constant feedback and clarification to the learners

11. Teaching online can be exciting because the instructor gets the opportunity to:
    a. work with new and emerging technologies

23(3) Summer 2010 Supplement
Educational Technology

1. Educational technology is best defined as?
   a. Current technology used to enhance education

2. The following educational technology tool utilizes the internet to synchronously stream both audio and video.
   b. Web conferencing

3. One of the most common reasons resulting in high attrition rates in online distance courses is
   a. Lack of communication

4. Which of the following educational technologies would benefit an instructor interested in delivering a series of short audio clips throughout the semester?
   a. Podcasting

5. An online course should begin with:
   b. Orientation

6. One of the interventions that helped to improve online student scores was
   b. Answering questions by interactive video

7. Providing an impetus to study earlier was associated with
   d. Tying the course objectives to the study guides

8. When reviewing with students, online faculty can
   c. Improve comprehension by using interactive technology

9. No single laboratory at UTMB affiliates does all of the immunology testing. This could explain the
   a. Difficulty in reinforcing concepts

10. In which of the following areas were statistically significant differences seen between the scores of on-campus and online students in 2008?
    b. Microbiology and Urinalysis

23(4) Fall 2010
Molecular Methods in Clinical Microbiology

1. Which DNA polymerase enzyme was used in the original PCR procedure?
   b. Klenow fragment of *E. coli*

2. Which molecular probes contain complementary sequences at the end forming a hairpin loop structure and use FRET technology to measure the intensity of the fluorescent signal generated?
   d. Hybridization probes

3. Agarose gels for detection of DNA amplicons are used in this reaction:
   c. End-point PCR

4. Which manufacturer markets Transcription Mediated Amplification and Hybridization Protection Assay methodologies for amplification and detection of HIV-1?
   d. Gen-Probe Inc.

5. If the viral load of an HPV assay was reported as .085 RLU/CO, the result would be considered a:
   d. False negative

6. The NucliSens HIV-1 Quantification assay manufactured by bioMerieux Inc., utilizes the following molecular methodology:
   c. NASBA

7. Which methodology utilizes signal amplification and chemiluminescent detection?
   b. Hybrid Capture

8. Which system manufactured by Cepheid can accommodate up to 48 modules for diagnostic testing?
   b. GeneXpert® Infinity System

9. The turn around time for the Xpert Group B Strep assay is approximately:
   a. 30 minutes

10. Which manufacturer markets a molecular detection system, which is used mainly in reference and research facilities and can not only run home brew assays but other manufacturer's assays as well?
    d. Cepheid

11. The COBAS AMPLICOR *Chlamydia trachomatis* / *Neisseria gonorrhoeae* test utilizes which methodology for amplicon detection?
    c. Colorimetric

12. TMA and HPA are methodologies used to identify which organism(s)?
    d. Mycobacterium tuberculosis

13. Cepheid's Xpert MRSA assay utilizes which molecular methodology?
    b. Real-time PCR

14. Biotin and avidin-horseradish peroxidase are reagents used in which assay?
    a. COBAS AMPLICOR CT/NG assay

15. Which methodology is used when avian samples are sent to the public health lab for WVN analysis?
    c. Real-time PCR

16. The turn around time using the 7900HT thermocycler in rapid mode (384 well format) is approximately:
    b. 52 minutes

17. Anti-rabies antibodies are labeled with this fluorochrome in the DFA test.
    c. Fluorescein isothiocyanante

18. Agarose plugs and restriction enzymes are required components in this methodology.
    d. PFGE

19. The medium used in the Mycobacteria Growth Indicator Tube is:
    d. Middlebrook 7H9

20. Multiplex PCR is used to identify the various serotypes of this organism:
    c. Strep pneumonia

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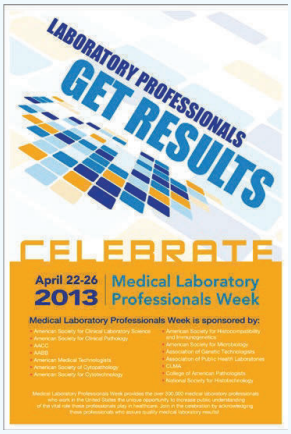
100% preshrunk heavyweight cotton.
White. **#25197**
$14.99 S-XL; $17.99 2XL-4XL

Ⓑ Women's Fleece Vest
100% polyester microfleece.
Royal, Fuchsia, Black.
#27644
$29.99 XS-XL; $32.99 2XL-3XL

Ⓒ Women's T-Shirt
100% preshrunk cotton.
Royal.
#25110
$9.99 S-XL; $12.99 2XL

Ⓓ Men's T-Shirt
100% preshrunk cotton.
Black.
#25108
$9.99 S-XL; $12.99 2XL-3XL



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