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ANSWERS TO 2007 CONTINUING EDUCATION QUESTIONS
Letter to the Editor

RE: CLIN LAB SCI 21(2) DD BECK
July 12, 2008

Dear Fellow Laboratorians:

I commend everyone on an excellent proposal. It is well thought out and the recommendation and levels of practice skills are exactly what this profession needs. I believe the proposed model is a step in the right direction. Unfortunately, I do not see an easy way to implement it without legislative incentives. The proposed recommendations to implement the practice are excellent, but why would anyone change now? Laboratory managers cater to administration and are unable to find the staff they need to fill positions. They are in a fight to get through each day.

Laboratory practitioners are looking at how green the grass is for all the other professions in the hospital and getting out of the laboratory. Administration does not support education needs of the laboratory staff.

There are so many laboratory certification agencies that no one, especially human resources, knows who is credible. This is one area that needs to be fixed before any real changes will occur in the laboratory. One organization should represent the laboratory, and they should support the proposal.

At the critical access hospital where I recently worked, we lost all the BSMTs capable of doing microbiology. Now only MLTs are available. There is a huge workload because the hospital is part of a system and one of the other small hospitals sends their microbiology there. This is a cash cow for the small hospital. Without a legislative mandate to hire a BSMT, I do not see this hospital changing. Of all the recommendations in the proposal, the one I support more than any other is having a Baccalaureate degree person in microbiology. I shudder to think how much is missed in this laboratory due to lack of education. I also wonder how many other rural laboratories will be able to meet the education level for microbiology. It is almost impossible to hire a med tech to work in microbiology in a rural laboratory. I do not see how they can comply with this proposal.

I am passionate about laboratory medicine and all that the great MLTs and MTs have done for their patients. I find it unfortunate that they are not respected and rewarded for all their hard work. I believe that licensure is the only way to make the changes your proposal suggests.

I have one last suggestion which is somewhat unrelated. Regarding Level V POC oversight, I caution anyone taking on the CLIA license for POC. I believe that the license should be held by the person accountable for the staff doing the tests. By that I mean if nursing is doing the majority of the POC testing, the nurse manager should hold the license. The Baccalaureate degree laboratory person should be an advisor. My concern is when I held the license (therefore I was legally accountable), I was unable to motivate the nursing staff to follow all the rules stated by CLIA and JCAHO.

Jean A Burr MS MT(ASCP)
MLT and phlebotomy instructor
College of the Albemarle, Elizabeth City NC
jburr@albamarle.edu

ERRATA

On page 210, the sentence “Inherited deficiencies or defects of the plasma proteins that are involved in blood coagulation can lead to life long bleeding disorders, the severity of which is inversely proportional to the degree of factor deficiency” should be corrected to read, “Inherited deficiencies or defects of the plasma proteins that are involved in blood coagulation can lead to life long bleeding disorders, the severity of which is directly proportional to the degree of factor deficiency.”

On page 211, the title of Table 1 reads “Prevalence of factor deficiency in their homozygous states”. In most instances, VWD is autosomal dominant, and VIII and IX deficiency are sex-linked recessives that appear in the “hemizygous” state.

On page 227, section DETECTION OF ID AND IDA BY LABORATORY TESTING OF INFANTS AND TODDLERS, the sentence “A hemoglobin or hematocrit is then run and should have risen by at least 10 g/L or 0.30 L/L, respectively, to confirm ID” should be corrected to read, “A hemoglobin or hematocrit is then run and should have risen by at least 10 g/L or 0.03 L/L, respectively, to confirm ID.”

Clinical Laboratory Science regrets the errors.
Human Ehrlichiosis: A Case Study

JACK G SCHNEIDER

Ehrlichiosis is an infection of white blood cells that affects various mammals, including mice, cattle, dogs, and humans.¹ It was first reported in dogs in 1935, and the first human case was documented in the United States in 1986. Ehrlichia are obligate, intracellular bacteria that are transmitted by ticks to humans. They grow as a cluster (morula) in neutrophils (Anaplasma phagocytophilum and E. ewingii) and in monocytes (E. chaffeensis).²⁻³ The infection may cause prolonged fever and general aches, and is characterized by leukopenia, cytopenia, and elevated liver transaminases.⁴ In the first week of infection, ehrlichiae can be detected by finding intracellular aggregates on the blood/body fluid smears and various other laboratory findings. Immunofluorescent antibodies (IFA) titers and PCR are generally needed for confirmation and a definitive diagnosis. Early diagnosis is necessary as antibiotic treatment with doxycycline is very effective.³⁻⁵

ABBREVIATIONS: ALP = alkaline phosphatase; ALT, SGPT = alanine aminotransferase; AST, SGOT = aspartate aminotransferase; BUN = blood urea nitrogen; PTT = partial thromboplastin time; WBC = white blood cell count.

INDEX TERMS: amblyomma americanum; anaplasmosis; ehrlichiosis; ixodes scapularis.


Jack G Schneider BS MT (ASCP) is of Clarian Health Partners/Indiana University School of Medicine, Division of Clinical Microbiology, Department of Pathology & Laboratory Medicine.

Address for correspondence: Jack G Schneider BS MT (ASCP), Clarian Pathology Laboratory, Clinical Laboratory Sciences Program, 350 W 11th Street, Indianapolis IN 46202. (812) 707-1773, (317) 491-6163 (fax). jgschneid@gmail.com

ACKNOWLEDGEMENTS: The author would like to acknowledge J Stephen Dumler MD, Division of Medical Microbiology, Department of Pathology, The Johns Hopkins Medical Institutions; Michael Sever MD, Indiana University School of Medicine, Department of Pathology and Laboratory Medicine; and the Indiana University Clinical Laboratory Sciences faculty at Indiana University School of Medicine.

CASE PRESENTATION

A 52-year-old avid outdoorsman has had multiple tick bites throughout his adult life. He experienced nonspecific malaise and fatigue for about one month. There were no fevers, chills, sweats, or weight loss. He was witnessed to have a generalized seizure and a marked decrease in mental status and was admitted into the hospital for thorough evaluation.

Due to sepsis-like presentation and clinical picture, he was treated empirically with broad-spectrum antibiotics, including vancomycin and doxycycline. Serum creatinine, blood urea nitrogen (BUN) levels, and hepatic enzymes were extremely high as a result of the patient’s acute renal and liver failure respectively. As his illness progressed, pulmonary function diminished and mechanical ventilation was required, as was hemodialysis. Multiple serology tests revealed that the patient was negative for Rickettsia, Borrelia (Lyme disease), histoplasmosis, West Nile virus, and viral hepatitis.

Because of his history of frequent tick bites, doxycycline was initiated upon admission and continued throughout his work-up. Further evaluation revealed positive serology for Ehrlichia chaffeensis with an initial titer of 64 during the first week’s test, 128 for the second week, and a maximum of 1024 by the third week.

The patient was eventually extubated and made an outstanding recovery. By the time of discharge, he was fully cognizant with clear thinking and normal speech. Liver function tests
improved and hematologic parameters returned to normal levels. Creatinine and BUN levels dropped to 2.7 and 44 respectively after the first week of dialysis. Hemodialysis was again implemented following discharge due to elevated BUN (84) and creatinine (7.0) (Table 1).

**History of the disease**
Since the end of the 20th century, several tick-borne diseases have been identified throughout the United States, including babesiosis, Lyme disease, and ehrlichiosis.1 Initially recognized in 1935, ehrlichiosis is caused by bacterial species in the genus *Ehrlichia*. Over time, veterinary pathogens within this genus were found to cause disease in a variety of animals. Currently, one species in Japan and three in the United States are known to cause disease in humans.1

With the advent of new diagnostic methods, such as PCR and gene sequencing, other disease-causing species of *Ehrlichia* may be identified in the near future.

The genus *Ehrlichia* was named after the German bacteriologist Paul Ehrlich, who surprisingly did not contribute to its classification and discovery. It is a more recently recognized disease, with the first diagnosed case in the United States occurring in 1986 in a 51-year-old man from Detroit who had been exposed to ticks in a rural area of Arkansas.1,6,7 Prior to this finding, the first ehrlichial pathogen was identified in Japan as causing Sennetsu Fever. This disease, caused by

Table 1. Selected laboratory data from patient on admission and discharge

<table>
<thead>
<tr>
<th></th>
<th>Patient admission laboratory values</th>
<th>Patient discharge laboratory values</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>$3.2 \times 10^9$/L</td>
<td>$6.5 \times 10^9$/L</td>
<td>$4.5-11.5 \times 10^9$/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10.0 g/dL</td>
<td>10.2 g/dL</td>
<td>14-18 g/dL</td>
</tr>
<tr>
<td>Platelets</td>
<td>$60 \times 10^9$/L</td>
<td>$230 \times 10^9$/L</td>
<td>$150-450 \times 10^9$/L</td>
</tr>
<tr>
<td>Partial thromboplastin time (PTT)</td>
<td>60.5 seconds</td>
<td>31.3 seconds</td>
<td>20-35 seconds</td>
</tr>
<tr>
<td>BUN</td>
<td>122 mg/dL</td>
<td>84 mg/dL</td>
<td>6-20 mg/dL</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>9.6 mg/dL</td>
<td>7.0 mg/dL</td>
<td>0.7-1.3 mg/dL</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST, SGOT)</td>
<td>1334 U/L</td>
<td>40 U/L</td>
<td>8-20 U/L</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT, SGPT)</td>
<td>300 U/L</td>
<td>77 U/L</td>
<td>10-40 U/L</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>274 U/L</td>
<td>360 U/L</td>
<td>53-128 U/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.8 g/dL</td>
<td>1.5 g/dL</td>
<td>3.5-5.2 g/dL</td>
</tr>
</tbody>
</table>
Neorickettsia (Ehrlichia) sennetsu, is characterized by swollen lymph nodes and fever. Most cases involving this species are very rare outside East and Southeast Asia and have only been reported in parts of western Japan and Malaysia.\textsuperscript{1,2}

Ehrlichiosis species represent a group of clinically similar, yet etiologically and epidemiologically distinct, diseases caused by \textit{A. phagocytophilum}, \textit{E. ewingii}, and \textit{E. chaffeensis}. The former \textit{Ehrlichia phagocytophila} is an organism that is similar to \textit{E. equi} and is now referred to as \textit{Anaplasma phagocytophilum} (which will be used throughout the rest of the discussion).\textsuperscript{1,2,3} The remainder of this paper will focus on the types of ehrlichiosis that occur in the United States, along with common treatments and preventative measures.

\textbf{Bacteriology/taxonomy}

The \textit{Ehrlichia} and \textit{Anaplasma} genera are classified as small, gram-negative, and obligate intracellular cocci that infect leukocytes in humans and various animal species. They range from 1 to 3 µm in diameter and generally divide within white blood cells to form morulae, characteristic of this bacterial pathogen.\textsuperscript{1,4,8}

These genera are found in the Anaplasmataceae family and include the following recognized species relevant to human infection: \textit{E. ewingii}, \textit{A. phagocytophilum}, and \textit{E. chaffeensis}. Initially these species were classified according to the blood cell most commonly infected (i.e., monocyte or granulocyte). However, it has been found that more than one species may be responsible for the generic clinical presentation called “ehrlichiosis”.\textsuperscript{1,4}

\textbf{Epidemiology and ecology}

Through 2003, the CDC recorded at least 1508 cases of Human Monocytic Ehrlichiosis (HME) in the United States, yet there may be underreporting since the disease is not reportable in all states. Endemic regions, such as southeastern Missouri, estimated the annual incidence rates of HME to be as high as 158 cases per 100,000 population.\textsuperscript{9}

The occurrences of these diseases seem to reflect the geographic distributions and seasonal activities of the tick vectors. Areas of the southeast, south-central, and mid-Atlantic regions correspond to places where \textit{Amblyomma americanum} (Lone Star) ticks, the vector of \textit{E. chaffeensis}, are plentiful. Most infections occur between May and July, which correspond to the peak feeding times of Lone Star ticks. Transmission occurs most often after bites of adult ticks, which generally are painless and leave no inflammatory skin lesion. This finding is quite different than in patients with Lyme disease where tick bites (\textit{Ixodes scapularis}) tend to cause inflammation, followed by radial extensions (erythema migrans).\textsuperscript{1,9}

Ehrlichiosis infection is quite distinct from several other tick-transmitted diseases in that it generally infects adults greater than 40 years of age, unlike Lyme disease and Rocky Mountain spotted fever, which occur often in children. Men generally are diagnosed with HME more frequently than women by a near ratio of two to one, possibly due to occupational and recreational exposure. Even though the average age of infection is 48, fatalities have also been reported in children.\textsuperscript{1,9}

\textbf{PATHOPHYSIOLOGY}

\textbf{Means of replication}

Generally, the \textit{Ehrlichia} species tend to infect leukocytes, where they enter the cell by endocytosis and once inside the host cell, they inhibit the fusion of the phagosome and lysosome. They develop within the host cell vacuoles first as reticulate cells and then as dense-core cells, eventually being released by lysis of the cell. The inclusion body that contains the organism is called a morula, due to its "mulberry-shaped" appearance (Figure 1).\textsuperscript{1,9,10}

\textbf{Human Monocytic Ehrlichiosis}

Caused by \textit{E. chaffeensis}, animals in the southeastern and mid-Atlantic United States such as white-tailed deer, dogs, 

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image1.png}
\caption{Anaplasma}
\end{figure}

\begin{flushright}
\end{flushright}
and foxes tend to serve as the animal hosts of this species. *E. chaffeensis* predominantly affects monocytes and macrophages, but it may also be seen rarely in the granulocytes of some patients with severe disease. The disease resembles Rocky Mountain spotted fever, except that a rash does not develop in over 67% of the cases.\(^{6,10}\)

**Human Granulocytic Anaplasmosis (HGA, formerly Granulocytic Ehrlichiosis-HGE)**

*Anaplasma phagocytophilum* is the causative agent of this disease and is carried by the deer tick (*Ixodes scapularis*). Common animal hosts throughout the spring and summer include the white-footed mouse, among many other mammals. The pathogen that causes HGA primarily infects granulocytes (mainly neutrophils and rarely eosinophils) and is distributed in the upper Midwest and northeast United States.\(^7,11\) This disease is similar to Human Monocytic Ehrlichiosis, except that mortality rates may be 10% higher.\(^{10}\)

**Ehrlichiosis Ewingii**

This disease is named for its causative agent, *E. ewingii*, and is also carried by the Lone Star tick. Like HME, its animal hosts include white-tailed deer, dogs, and foxes throughout the spring and summer. It is commonly found in the south-eastern, south-central, and mid-Atlantic United States, and primarily infects neutrophils and occasionally eosinophils.\(^9-10\) The symptoms and signs are similar to HME and HGA, but infected patients usually have pre-existing medical conditions that cause immunosuppression such as splenectomies, HIV, and transplantations.\(^1\)

**CLINICAL FEATURES**

**Signs and symptoms**

A person infected with ehrlichiosis generally presents with nonspecific signs and symptoms. In fact, it is possible that many infected people do not even seek treatment because the development of the illness may be mild and display no symptoms.\(^1\)

Initial manifestation of infected patients commonly include fever, headaches, malaise, and muscle aches after an incubation period of approximately 5 to 10 days after the tick bite.\(^1\) Other signs and symptoms include nausea, vomiting, cough, confusion, and occasionally a rash. Rashes are uncommon in adult patients with HME and HGA, but 60% or more of infected pediatric patients display this rash when infected with *E. chaffeensis*. Because most patients with ehrlichiosis present with fever and a severe headache, meningitis may be in the differential diagnosis.\(^1,9\) The CSF profile in patients with ehrlichiosis is usually normal, but about 20% with signs of meningitis will have CSF pleocytosis similar to patients with viral or bacterial meningitis. Other diagnostic possibilities include malaria, babesiosis, Lyme disease, and Rocky Mountain spotted fever, suggested by a fitting epidemiological/travel history.\(^1,10\)

If left untreated, ehrlichiosis can become very severe and possibly lead to renal failure, disseminated intravascular coagulation (DIC), respiratory distress, and prolonged fever. With *E. chaffeensis* infection being the most common and most severe, only two to three percent of patients actually die from this infection.\(^{14}\)

Overall, the seriousness of HME is dependent on the immune status of the patient. Corticosteroid therapy, chemotherapy, and HIV infection are examples that may put the patient at a greater risk of fatal outcomes.\(^1,10,12\)

**Laboratory detection**

Availability of confirmatory assays presents a huge challenge in diagnosing ehrlichial infections. Therefore, doctors tend to make their treatment decisions based on clinical and epidemiologic clues. In general, clinical laboratory tests supportive of ehrlichiosis include a low white blood cell count, low platelets, and elevated liver enzymes. The biggest clue is the peripheral blood smears containing morulae in the leukocytes on a Giemsa stain.\(^1,9,10\)

Leukopenia, which involves decreased numbers in both neutrophils and lymphocytes, is usually resolved after the first few weeks of illness with proper treatment. The cytopenias cannot be explained by bacterial lysis of infected cells because they do not infect erythrocytes and platelets. However, the frequent detection of macrophage-rich inflammatory infiltrates within the liver, often along with hemophagocyte cells, suggests macrophage activation as the mechanism for both the cytopenias and the frequency of increased serum transaminases (ALT and AST).\(^8,13,14\)

Standard media used in the microbiology laboratory is generally not successful in isolating Anaplasmataceae (*Anaplasma* and *Ehrlichia*). Therefore, laboratory confirmation requires serologic, molecular, or cell culture based methods. Serologic evaluations use indirect immunofluorescence assays (IFA), but these may not always detect the organism. Most patients demonstrate increased IgM or IgG titers by the second week of the illness, and IgG levels usually stay elevated up to 2.5 years after the acute illness. One disadvantage of this testing...
is the likelihood of cross reactivity between the different species, hindering the epidemiological distinction between the ehrlichial infections.\textsuperscript{1}

Polymerase chain reaction (PCR) may be used to detect the organism. The detection of \textit{E. chaffeensis} DNA is based upon the amplification of specific genomic DNA sequences. The diagnosis of infection should not rely solely upon the PCR assay; however, a positive PCR result should be considered in conjunction with clinical presentation and additional diagnostic tests.\textsuperscript{1,9,15} Above all, direct isolation is the most desired method but can be very time-consuming. \textit{E. chaffeensis} and the \textit{A. phagocytophilum} have been isolated from blood samples when inoculated to canine DH82 or human HL-60 cells. The organism usually grows within 7 to 36 days.\textsuperscript{1}

**Diagnosis**

The diagnosis of ehrlichiosis requires not only laboratory evidence, but also a well-matched clinical history characteristic to the disease. Significant laboratory evidence includes a single IgG antibody titer of at least 256, a 4-fold rise in titer between the acute and convalescent stage, recovery of \textit{E. chaffeensis} or \textit{A. phagocytophilum} in culture, or detection of their nucleic acids in blood by PCR. These specific tests, along with common laboratory values and characteristics, help the physician to determine a definitive diagnosis. The patient case history previously described demonstrates why physicians should consider ehrlichiosis when patients present with fever, cytopenias, myalgia, leukopenia, thrombocytopenia, elevated liver enzymes, and above all, a history of tick bites or exposure.\textsuperscript{1,4,6,9-10,15}

**Treatment/prognosis**

Most patients with ehrlichiosis respond favorably to treatment, particularly if the therapy is initiated early in the disease. Doxycycline is the antibiotic of choice and within 24 to 72 hours after treatment, fever generally goes down. After one week, white blood cell and platelet counts usually return to normal. Fatigue may persist for weeks to months, but depending on the health status of the individual, recovery time is generally short.\textsuperscript{1,9-10}

**Prevention and control**

Ehrlichiae is spread by tick bites. Therefore, limiting one’s exposure to ticks reduces the chances of infection. Common measures include using insect repellent, wearing clothing to

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**Table 2. Selected epidemiological and ecological features of ehrlichioses\textsuperscript{9}**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Human Monocytic Ehrlichiosis (HME)</th>
<th>Human Anaplasmosis (Granulocytic Ehrlichiosis)</th>
<th>Ehrlichiosis “Ewingii”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector</td>
<td>Lone Star tick (\textit{Amblyomma americanum})</td>
<td>Deer tick/black-legged tick (\textit{Ixodes scapularis})</td>
<td>Lone Star tick (\textit{Amblyomma americanum})</td>
</tr>
<tr>
<td>Animal host</td>
<td>White-tailed deer, dogs, foxes, wolves</td>
<td>White-footed mouse, other mammals</td>
<td>White-tailed deer, dogs, foxes, wolves</td>
</tr>
<tr>
<td>Cells infected</td>
<td>Monocytes</td>
<td>Neutrophils</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Time of year</td>
<td>Spring/summer</td>
<td>Spring/summer</td>
<td>Spring/summer</td>
</tr>
<tr>
<td>Location</td>
<td>Southeastern, south-central, and mid-Atlantic US</td>
<td>Upper Midwest and northeast US, California, and Europe</td>
<td>Southeastern, south-central and mid-Atlantic US</td>
</tr>
</tbody>
</table>
cover skin, avoiding dense brush and long grasses, and constantly checking for ticks and removing any that be found on the body after being outside, especially in endemic areas. Studies have shown that a tick must be attached to the body for at least 24 hours in order to cause disease, so early removal may prevent the spread of infection (Table 2). \(^{1,10,12,16}\)

**CONCLUSION**

Human ehrlichiosis is a rising disease that generally provides few clinical clues for diagnosis and may cause severe infections or even death in infected patients. It is imperative to be mindful of this disease and acknowledge common risk factors associated with it, such as traveling to endemic areas and tick bites. Fever, elevated liver enzymes, and cytopenias are all important laboratory findings when dealing with ehrlichiosis. Even if suspected patients are unaware of vector contact or merely traveling in endemic areas, administering doxycycline therapy is the best treatment when ehrlichiosis is suspected but not yet confirmed. \(^{8,11,13}\)

*Clin Lab Sci* encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to brodak@iupui.edu. In the subject line, please type “CLIN LAB SCI 22(1) CP SCHNEIDER”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

**REFERENCES**


The ability of a clinical laboratory scientist (CLS) to perform molecular diagnostic testing has become critical to the profession. Knowledge of methodology associated with detection of pathogens and inherited genetic disorders is imperative for the current and future CLS. CLS programs in the US teach human genetics and molecular diagnostics in various components and formats. Integrating these sometimes expensive methods into the curriculum can be challenging. This article provides a commentary with specific details associated with our experience in designing a dedicated CLS molecular diagnostics course. It offers a flexible template for incorporating a lecture and laboratory course to address theoretical and practical knowledge in this dynamic area of the laboratory.

ABBREVIATIONS: ACMG = American College of Medical Genetics; ASCLS = American Society for Clinical Laboratory Science; CAP = College of American Pathology; CLIA = Clinical Laboratory Improvement Amendment; CLSI (formerly NCCLS) = Clinical and Laboratory Standards Institute; CLS = clinical laboratory science; MD = molecular diagnostics; NAACLS = National Accrediting Agency for Clinical Laboratory Science; PCR = polymerase chain reaction; QA = quality assurance; QC = quality control.

INDEX TERMS: clinical laboratory science; education methods; molecular diagnostics; teaching techniques.


The peer-reviewed Clinical Practice Section seeks to publish case studies, reports, and articles that are immediately useful, are of a practical nature, or contain information that could lead to improvement in the quality of the clinical laboratory's contribution to patient care, including brief reviews of books, computer programs, audiovisual materials, or other materials of interest to readers. Direct all inquiries to Bernardette Rodak MS CLS(NCA), Clin Lab Sci Clinical Practice Editor, Clinical Laboratory Science Program, Indiana University, Clarian Pathology Laboratory, 350 West 11th Street, 6002F, Indianapolis IN 46202. brodak@iupui.edu

Rodney E Rohde MS, SV, SM, MP (ASCP)CM is associate professor; David M Falleur Med MT(ASCP) CLS(NCA) is associate professor and chair; and Phil Kostroun Med MT (ASCP) is associate professor (retired); Clinical Laboratory Science Program, Texas State University – San Marcos, San Marcos TX.

Address for correspondence: Rodney E Rohde MS, SV, SM, MP (ASCP)CM, associate professor, Clinical Laboratory Science Program, Texas State University – San Marcos, HPB 361, 601 University Drive, San Marcos TX 78666-4616. (512) 245-2562, (512) 245-7860 (fax). rrohde@txstate.edu.

ACKNOWLEDGEMENTS: The authors would like to thank Sam Sutton (President, Embark Scientific, Austin TX) and Lisa Sutton (Vice President, Embark Scientific) for their collaborative support in the development of the laboratory component of this molecular diagnostics course and for assisting in the development of this article. We also thank them for sharing their expertise in clinical molecular diagnostics (http://www.embarkscientific.com/) with our former, current, and future CLS students.

Personnel in clinical laboratories around the world are being asked to provide rapid identification of emerging and reemerging disease-causing agents associated with both “common” disorders and bioterrorism preparedness activities. The clinical laboratory has always been an evolving environment in which personnel are constantly challenged to implement new diagnostic tests designed to provide more sensitive and specific tests for detecting and monitoring disease.1 Clinical laboratory scientists (CLS) are being challenged yet again by the introduction of complex molecular diagnostic techniques that were formerly performed only in research settings. Historically, the prevention, control, and treatment of infectious diseases are improved by early and accurate identification of the causative pathogenic organism. Many detection procedures require the pathogen to be grown in culture, followed by analytical testing in differential media for proper identification. These tests, although usually effective, can be slow and costly. Further, the organisms (especially bacteria and parasites) can be fastidious or cannot be cultivated at all, leading to severe limitations in pathogen detection, and ultimately, delayed patient treatment. To overcome these
major constraints, molecular diagnostic (MD) techniques are being developed and introduced into routine laboratory practice. For a MD approach to succeed in a clinical setting, it is critical that CLS, residents, and clinicians be well trained in performing, troubleshooting, and interpreting the assays. They must understand the limitations (e.g., false positives, false negatives, cross-reactivity, contamination issues, and inhibition of amplification) of both the technology and the results produced from MD tests.

MD testing has shifted dramatically in the past decade from the research arena to the clinical arena. The success of the Human Genome Project, forensic applications, genetic identification of various disease-causing microbes, establishment of the Laboratory Response Network (LRN) for detection of bioterrorism agents, and expanded public health epidemiology and surveillance activities have all contributed to the incorporation of MD into the routine practices of medical and public health laboratories at a rapid pace. CLS programs in the US have been asked to educate the future laboratory professional with the goal of being knowledgeable in the basic principles and uses of MD technology. An informal telephone survey in 1992 of CLS program directors and a formal survey in 1993 indicated that only 8% to 16% of the responding programs required a genetics course as part of their curriculum or as a prerequisite. A more recent survey in 2002 of 263 CLS programs indicated that over 92% of programs teach human genetics and MD in varied formats. Briefly, this survey found that more programs teach theory than hands-on wet laboratories. Importantly, there was noted dissatisfaction in the education provided in the MD area with respect to time issues, lack of knowledgeable faculty, associated costs, and implementation.

The following is a review of how the CLS program at Texas State University – San Marcos introduced a dedicated MD course (lecture/laboratory) in the spring of 2002. This overview focuses on the dynamic nature of the course resources that are used to teach the concepts of MD and prepare CLS students for entry level skills. Students are required to take a prerequisite genetics course prior to the CLS MD course. During the spring semester of the senior CLS academic year, the students receive dedicated MD didactic lectures and laboratory exercises to achieve active learning. MD topics are also intermittently discussed throughout the two year curriculum with specific topics (e.g., Gen Probe assays in microbiology, Factor V Leiden deficiency in hematology, viral load and genotyping for HIV in immunology, etc.). The evolution of lecture and laboratory components of this dedicated course will also be discussed.

RESOURCES FOR MOLECULAR DIAGNOSTICS

With the lack of specific guidelines for molecular testing in the Clinical Laboratory Improvement Amendments (CLIA) final rule, one must turn to the recommendations of the National Accrediting Agency for Clinical Laboratory Science (NAACLS), American College of Medical Genetics (ACMG), Clinical and Laboratory Standards Institute (CLSI) and College of American Pathology (CAP). NAA-CLS describes the programmatic accreditation process for the institution of the diagnostic molecular scientist by providing competencies and requirements for this professional. CLIA defines many of the basic quality systems required for laboratories, but lack specific guidelines pertaining to molecular genetic testing. These standards and practice guidelines can be applied to many areas of molecular testing regardless of the field of study. The ACMG Standards and Guidelines cover cytogentic, biochemical genetics, and molecular genetics. In addition, the ACMG has developed disease-specific guidelines to address specific technical problems frequently seen in complex assays. Together, these guidelines cover general laboratory practices, assay validation, and method-specific and disease-specific technical issues.

The Clinical and Laboratory Standards Institute (CLSI), formerly known as National Committee on Clinical Laboratory Standards (NCCLS), used field-specific experts to develop a number of guidelines for molecular diagnostics, including molecular genetic testing, molecular hematopathology, DNA sequencing, diagnostic microarrays, and proficiency testing. The College of American Pathologists (CAP) is the main accrediting organization for molecular laboratories. The inspection checklists for General Laboratory and Molecular Pathology are good references for the type of quality systems and procedures that should be operating in a molecular laboratory. These documents provide expert opinions on standard practices in a molecular laboratory. They offer guidance on specific techniques and appropriate controls. Furthermore, molecular testing is becoming more complex as an increasing number of analytes are now being measured on microarray platforms. In addition to the CLSI guidelines for microarrays, the ACMG is working on guidelines for genomic microarrays. These guidelines will continue to be developed and updated as more applications of microarrays move into clinical practice. Importantly, the position of ASCLS is that “the profession (of a CLS) includes generalists as well as individuals qualified in a number of specialized areas of expertise” including MD.
IMPLEMENTATION OF MD IN THE CLS CURRICULUM

Didactic lectures

While some have integrated MD topics and laboratories in different CLS courses throughout the curriculum, a dedicated MD lecture/laboratory course was introduced at Texas State University in 2002. The faculty at Texas State University takes advantage of the opportunity to present appropriate MD clinical applications in other courses (e.g., viral load and genotyping for HIV in immunology); however, an immersion in a dedicated course is critical to allow for deeper learning and understanding of the content. A variety of textbooks has been utilized for this course and is listed in Table 1. Regardless of the textbook, the topics selected in the lecture have remained fairly stable and are listed in Table 2.

The course begins with a review of molecular biology “basics” (central dogma) surrounding DNA, RNA, and proteins. The lecture topics follow with extraction/isolation techniques, amplification techniques such as polymerase chain reaction (PCR), and mutation events. The latter topics introduce post-analytic techniques (e.g., sequencing) and detection (inherited disorders, infectious disease, oncology), and conclude with quality assurance and control (QC/QA) in MD. The lectures are supplemented with case studies associated with MD data sets (gel images, dendograms) and problem sets requiring student calculations (concentration, purity, primer design). Students are evaluated on the material based on their answers to problem sets, case studies, and several unit exams.

A special assignment for course credit involves student groups conducting a literature review of a MD technology as it applies to a “real world” CLS case. This assignment coincides with our College of Health Profession Faculty – Student Research Forum. Students are required to submit an abstract detailing their topics with a final poster presentation.

Table 1. Textbook resources for MD course

<table>
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<tr>
<th>Textbook resource</th>
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Table 2. Topics for molecular diagnostic (MD) lectures

<table>
<thead>
<tr>
<th>Unit number and topics</th>
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<tbody>
<tr>
<td>1. DNA: An Overview</td>
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<td>2. RNA &amp; Proteins: An Overview</td>
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<tr>
<td>3. Nucleic Acid Extraction Methods</td>
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<td>4. Resolution, Detection, &amp; Analysis</td>
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<tr>
<td>5. MD Amplification</td>
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<tr>
<td>• PCR</td>
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<td>• qPCR</td>
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<tr>
<td>• Reverse-transcriptase PCR</td>
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<tr>
<td>6. Chromosomal Structure &amp; Mutations</td>
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<td>7. Gene Mutations</td>
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<tr>
<td>8. DNA Sequencing</td>
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<tr>
<td>9. DNA Polymorphisms &amp; Human Identity</td>
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<tr>
<td>10. Detection &amp; Identity of Microbes</td>
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<tr>
<td>11. Detection of Inherited Diseases</td>
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<tr>
<td>12. Molecular Oncology</td>
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<td>13. QA &amp; QC</td>
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<tr>
<td>• Agency oversight</td>
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<td>• Regulations</td>
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Laboratory component
The laboratory component of the MD course is taught concurrently with the didactic component. Concurrent lecture and laboratory sessions allow the student to be involved in the actual generation of data using clinically relevant MD tools and techniques. Senior students are also beginning their clinical rotations in various community laboratories during this semester which permits possible observation and experience with MD equipment and methods in the hospital and reference laboratory setting. Finally, the concurrent MD laboratory helps reduce the problem of lecture topics becoming abstract or distant before the student has an opportunity to “practice” what’s being covered in the didactic lecture.

During the initiation of the course, the laboratory component was a mixture of online “virtual” experiences from a variety of websites (e.g., DNA from the Beginning at http://www.dnaftb.org/dnaftb/, Cold Spring Harbor Laboratory Dolan DNA Learning Center at http://www.dnalc.org/ddnalc/about/) or training CDs from the Roche Education Program (e.g., Genetics, Hepatitis C, MRSA/ VRE, Regulatory and Molecular Technology). These virtual laboratory experiences were followed by selected molecular kits (available from a variety of suppliers) covering areas such as (1) isolating the student’s DNA from cheek cells with PCR of particular genes, (2) mock crime scene investigation with PCR and restriction fragment analysis, and (3) PCR of known controls. Each of these modules incorporated the use of typical extraction kits, PCR thermocyclers, and post PCR work via gel electrophoresis and analysis of products.

In 2008 we collaborated with a local biotechnology company (Embark Scientific, Austin TX) to incorporate a “beginning to ending” real world application of clinical MD in our laboratory component. The specific exercise was planned to integrate the application over the entire semester between student clinical rotations. The assay design process was discussed broadly with the students with respect to mecA and femA genes in Methicillin-resistant Staphylococcus aureus (MRSA) and for the iroB gene in Salmonella enterica. The iroB gene was chosen for the laboratory exercise because it has been extensively documented in the literature. Sequence data for the iroB gene was obtained at the National Center for Biotechnology (NCBI) website (http://www.ncbi.nlm.nih.gov/) and publication for utilization of specific primers and for student exercises in primer design techniques.

Each student was provided an overnight culture of Salmonella enterica (Central Texas Medical Center, San Marcos TX) for total DNA isolation/purification using the DNeasy Blood and Tissue kit (Qiagen Inc., Valencia CA). PCR design and troubleshooting was reviewed and each student performed PCR amplification of iroB utilizing primer iroB F1: TGGACTGCTATACCTGTGC and primer iroB R1: GCAGTATGCTCATGCTGGGC which yields a 493bp PCR fragment. Primers (Integrated DNA Technologies, Inc., Coralville IA) were resuspended at 100µM. The DNA template (3µL) was added to a PCR mixture (Promega, Madison WI) with a total volume of 50µL and a final MgCl₂ concentration of 2mM. PCR cycling was performed as recommended by Embark Scientific. PCR products and DNA markers (Amresco Inc., Solon OH) were separated by agarose gel electrophoresis (Amresco Inc., Solon OH) to confirm correct iroB gene size (493bp) with photodocumention (Figure 1).
PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland OH) to provide clean template for DNA sequencing. Sequencing reactions were prepared by adding 5.0µL of each sample or positive control (pUC19), 2.0µL sequencing primers (1.6uM), 8.0µL DTCS Quick Start Master Mix and ddH2O for a total volume of 20µL (Beckman Coulter, Inc., Fullerton CA). Sequencing reaction amplification was performed with 30 cycles of one minute at 94°C, 20 seconds at 96°C, 20 seconds at 50°C and four minutes at 60°C. Sequencing reaction cleanup by ETOH precipitation was performed on final products.

DNA sequencing was performed using a capillary array instrument (Beckman Coulter Genomic Analyzer acquired by R Rohde via an Education/Research Grant from Beckman Coulter and matching grant from Texas State University). Students were instructed on how to create databases and project folders to manage the sequencing data and to enter necessary sample information into the instrument software program. Instrument startup and shut down procedures were performed including: installing, removing or replacing Capillary Arrays, installing Gel Cartridges, priming the system (Manifold Purge, Gel Capillary Fill and Optical Alignment), installing gel waste bottle, preparing plates for run, loading Wetting Tray, Sample Plate and Buffer Plate and system cleanup. Students exported sequencing data of their samples (Figure 2) and analyzed it utilizing typical sequencing analysis software.

This laboratory component is critical to the students’ understanding of mathematical operations (calculating DNA concentrations, PCR master mix) and interpretation of data (troubleshooting gels, controls). Students are evaluated on technique by the instructor (pipetting, gel loading, maintenance of equipment, QA/QC) during the entire semester and are given a concluding final exam. The final exam includes theory of basic concepts and synthesis/critical problem solving of data interpretation (Table 3).

Clinical practicum
Due to the limitation of MD applications in the clinical laboratories in our geographic location, the students may spend time only in some laboratories doing MD assays and QC/QA of equipment associated with molecular techniques.

Table 3. Topics for molecular diagnostic (MD) laboratory

1. Assay Design: An Overview
   - Define/obtain target sequence
   - Primer design
2. DNA Isolation/Purification Methods
3. PCR Method: An Overview
   - Assay design
   - Reaction component interactions
   - Troubleshooting
   - PCR amplification
4. PCR Analysis
   - Agarose gel electrophoresis
   - Photodocumentation
5. PCR Cleanup Method
6. DNA Sequencing: An Overview
   - DNA sequencing reaction setup & amplification
   - DNA sequencing reaction cleanup procedure
   - Instrument setup & reaction run procedures
   - Instrument startup & shut down procedures
   - Data Management
7. DNA Sequence Analysis
   - Review analyzed data
   - Export data
   - Data analysis

Figure 1. Agarose gel of student PCR iroB gene amplicons (493bp)

DNA markers are shown on the far left and right lane and student samples (capital letters depict student samples) are between the DNA markers.
The clinical practicum is an area that we feel can be nurtured and grown with our area affiliate laboratories as they incorporate MD into their testing menus. It is important to mention that hospitals, upon finding out about our dedicated MD course, actively recruit our students to “help set up” that type of testing as future employees.

**SUMMARY**

Molecular diagnostics is the fastest growing area of clinical medicine. Current CLS students and working CLS professionals need to be proficient in this area of the laboratory. MD often allows for faster turnaround times with increased sensitivity and specificity. However, this testing must be integrated with strict QC/QA with respect to the types of controls, standards, and limits.\(^{16}\)

By including a dedicated MD course in the CLS curriculum, we are preparing our students with the knowledge and background they need to be competent in applying this skill set in the workforce. The course has strengthened our students’ “job attractiveness” in clinical, reference, research, and public health laboratories. The future of CLS students in MD has arrived and they need the strong background in this exploding diagnostic area of the medical world to effectively and accurately perform the growing number of FDA approved clinical testing platforms (e.g., cystic fibrosis, Factor V, and non-culturable microbes). Having a course in MD and gaining work experience in molecular techniques post-CLS degree also allows CLS professionals who meet the proper requirements to obtain certification from a variety of organizations, for example technologist in molecular pathology (MP) from ASCP.\(^{20}\)

It is important to mention the challenges associated with the endeavor of pursuing this type of course in the CLS curriculum. The major obstacles that we encountered were (1) dedicated space (clean area outside of typical routine CLS teaching laboratories), (2) faculty expertise, (3) time of placement within CLS curriculum, (4) student preparation for course rigor (prerequisites), (5) reagent and equipment cost, and (6) a MD clinical experience for the student.

These obstacles were addressed in a variety of ways. As we acquired funding and gained recognition within our College of Health Professions and university about the importance of this course, additional dedicated laboratory space was provided to support this course and the research expertise of the faculty member. Faculty expertise was met in the initial year (2002) of the course implementation by employing a new faculty member with MD skills. Subsequently, the faculty member augmented the laboratory component of the course by collaborating with a local biotechnology company (Embark Scientific) to assist in the course development from a real world perspective. The placement of the MD course in the curriculum and student preparation will be different for each CLS program. In our experience, the course was best placed in the senior year so that students would have the opportunity to finish prerequisites and build their skills in critical areas (pipetting, calculations, etc.). The financial costs associated with the course can be problematic. However, a program can reduce this issue by using home-brew assays, utilizing molecular equipment that is available in other departments on their respective campus (or laboratories with clinical affiliations), and using expired or donated kits from companies. Acquiring a MD clinical practicum or rotation for students is an ongoing challenge for our program. However, by reaching out to local biotechnology companies, research laboratories, and hospitals, we are finding more possible sites to place students for experience in molecular techniques.

A dedicated course in MD provides CLS programs with the unique opportunity to become flexible in the face of a growing clinical need. While molecular biology theory and general laboratory work is taught in a variety of university and college departments (biology, biochemistry, forensics), CLS programs can become the leader in preparing clinically competent CLS professionals by providing them with training in MD.

*Clin Lab Sci* encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to brodak@iupui.edu. In the subject line, please type “CLIN
LAB SCI 22(1) CP ROHDE”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

REFERENCES
**RESEARCH AND REPORTS**

**Student Perceptions of the Clinical Laboratory Science Profession**

KAREN MCCLURE

**OBJECTIVE:** The purpose of this paper is to describe the attitudes and perceptions among college biology and CLS/CLT students. These students were on selected college campuses at Texas universities in Houston, Dallas and the Austin/San Antonio areas for the Spring 2007 semester. Specifically, students were questioned on factors that influence their choice of field of study, career expectations, legislative measures which might be used to attract individuals to the career, and factors that will be required to keep them in the field of practice.

**DESIGN:** This study was part of a larger qualitative study which included exploratory discovery and inductive logic regarding the attitudes of four focus groups in Texas.

**SETTING:** Focus groups took place on college campuses or in hotel conference rooms.

**PARTICIPANTS:** 1) junior/senior-level college biology students and 2) junior/senior-level students currently enrolled in CLS/CLT programs.

**INTERVENTIONS:** Focus group discussions using a standard set of questions; group sessions lasted about 45 minutes.

**MAIN OUTCOME MEASURE:** This study was a qualitative study which included exploratory discovery and inductive logic regarding the attitudes of two groups in Texas.

**RESULTS:** College biology and CLS/CLT students find the clinical laboratory science profession to be interesting and exciting as a career prospect, however, many do not see themselves remaining in the profession and perceive it does not have good prospects for career advancement. The majority of students must work to support themselves through their college education and would welcome additional grants, scholarships and loan forgiveness programs as incentives to study the clinical laboratory sciences. Students believe that additional recruitment on high school and college campuses is needed to increase the visibility of the field as career choice.

**CONCLUSION:** The majority of students who are entering the clinical laboratory science profession do not see the profession as their final career choice, but rather a stepping stone to another career field in healthcare or a related field. The perception that the profession lacks a career ladder is a critical detriment to the retention of CLS/CLT professionals. The clinical laboratory science profession continues to suffer from a lack of knowledge about the field by the general public, college advisors, and even healthcare workers. State and national programs involving grants/scholarships or loan forgiveness programs offered by healthcare institutions would be beneficial in attracting students to study the clinical laboratory sciences.

**ABBREVIATIONS:** BLS = Bureau of Labor Statistics; CLS/CLT = clinical laboratory science/clinical laboratory technician; US = United States

**INDEX TERMS:** administration; behavioral research; health occupations; hospital; students.


Karen McClure PhD CLS(NCA) MT (ASCP)SBB is director of the Clinical Laboratory Science Program and assistant professor at the MD Anderson School of the Health Professions, Houston TX.

Address for correspondence: Karen McClure PhD CLS(NCA) MT(ASCP)SBB, 1100 Holcombe Blvd. Unit 204, Houston TX 77030. (713) 703-5292. kmclure@mdanderson.org.

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An adequate supply of clinical laboratory scientists (CLS) and technicians (CLT) is essential to achieving the nation’s goals of ensuring accessible, high quality healthcare at a reasonable price and being able to respond effectively to a bioterrorist event or a national healthcare emergency.1-11
The Bureau of Labor Statistics in its 2006-07 Occupations Outlook Handbook cites an estimated 68,700 new CLS/CLT positions will be added between 2004-2014 (average growth of 6,870 new positions annually).1,2,13 In 2005 a total of about 4,390 CLS/CLTs graduated from schools in the US.13

By focusing on those who are considering entering or soon will enter the field of practice, it was hoped that insights might be gained into what this generation of young adults are looking for in a career which would attract and retain them in this healthcare field.

According to the May 2006 BLS report, there are an estimated 25,720 CLS/CLTs working in Texas; 62% of the CLSs and 57% of the CLTs are working in the Dallas, Houston, and Austin/San Antonio areas.1,2,13 Thus, these metropolitan areas were targeted for focus group sessions.

The results of the BLS estimates and those from a Doig and Beck study both conclude that the nation is losing workers at a much quicker pace than it is replacing them.5,12 The anticipated national deficit can be estimated using the information on workforce numbers from the BLS and the Doig/Beck study (Figure 1). These numbers point to a 34% deficit in the number of clinical laboratory workers needed by 2015.

This lack of qualified workers in clinical laboratories will directly impact the efficiency and quality of patient care and impede appropriate actions in response to public health threats (e.g., bioterrorist events, emerging infectious diseases, etc.).2,4

The need to discover and understand the attitudes and perceptions towards a career in the clinical laboratory sciences, from those who will soon be entering the field, is a key element in understanding what is necessary to maintain the vitality of the CLS/CLT profession.

This study was a qualitative study which included exploratory discovery and inductive logic regarding the attitudes of two groups in Texas: 1) junior/senior-level college biology students and 2) junior/senior-level students currently enrolled in CLS/CLT programs. Exploratory discovery is used to understand the reason behind the numbers seen in quantitative studies by exploring the “what, why, and how” of people’s views. Inductive logic uses a repetitive group of observations to reach a general conclusion, which is most likely true, although not certain.14,15

This paper focuses on the results from two groups: the college biology students and CLS/CLT students. It was imperative that the members of the focus groups were representative of the target population from which the information was desired.14,15 It is also a comparative study looking at these two groups and their attitudes and perceptions on career selection factors, incentive measures which might attract individuals to an allied healthcare career, and the field of practice. It was postulated that there would be some areas of significant differences to the questions posed in the study between the two groups.

A discussion guide was developed containing the questions which focused on the perceptions and attitudes college biology and CLS/CLT students had towards a career in the clinical laboratory sciences; topics included the appeal of the professions, skills needed at entry level, impressions about the career, and what factors might be useful in attracting individuals to the profession.

There were a total of 56 research participants in six focus groups (three groups of college biology students and three groups of CLS/CLT students) conducted in three metropolitan locations.
within the state of Texas; Houston, Dallas, and Austin/San Antonio areas. Tables 1 and 2 contain demographic information about the groups upon which this paper is based.

This study was approved by the Committee for the Protection of Human Subjects at the University of Texas School of Public Health and a consent form was completed by each participant; additional Internal Review Board (IRB) approvals were obtained where necessary.

A type of boundary analysis was used to identify all the themes of the focus group discussions. Codes were assigned to broad themes upon review of the transcripts from the focus group; the transcripts were analyzed three times to ensure accurate documentation of the coding and thorough analysis of the information obtained. Each transcript was evaluated, line by line, to identify themes/patterns. As the themes developed, a code was assigned and used to evaluate the next transcript. As other themes/perspectives emerged, new codes were developed; new codes were added only if a group of statements did not fit into another, already identified category. Classificational analysis was used to logically divide the responses into classified themes.

All information that might compromise confidentiality was removed from the report prior to submission to any outside reader.

RESULTS
Attitudes and perception about a CLS career
Participants cited many reasons for the appeal of a career in clinical laboratory science, but the most often cited reasons across all focus groups were:
- laboratory setting offers a variety of work environments.
- knowledge gained; the ability to solve problems, understand how the body works.
- hands-on type of work, e.g., laboratory work.
- healthcare career with very little patient contact.

When asked to identify individuals who influenced their choice of career, the most often cited from both groups were self-directed Internet searches and teachers (high school advisors, CLS/CLT program representatives). College advisors were infrequently mentioned.

The most frequent comment students gave to the questions about impressions about a career in the clinical laboratory science centered on the lack of knowledge by others (other healthcare workers or the public) about CLS/CLT job duties. Some of the CLS/CLT students commented on the lack of respect CLS/CLTs received from nurses and physicians. Additional comments included:
- an exciting career, again citing diversity of work settings.
- salary not commensurate with knowledge needed to do the job.
- a position underutilized by the

| Table 1. Characteristics of focus groups: junior/senior-level college biology students |
|---------------------------------|-------------|-------------|-------------|-------------|
|                                | Houston (n=7) | Dallas (n=5) | Austin (n=8) | Summary percentage focus groups |
| Gender                         |              |              |              | Texas (2006 US Census data)    |
| Female                         | 5            | 4            | 7            | 80%                             |
| Male                           | 2            | 1            | 1            | 20%                             |
| Age (years)                    |              |              |              |                                 |
| 18-25                          | 4            | 4            | 7            | 75%                             |
| 26-30                          | 2            | 1            | 1            | 20%                             |
| 31-35                          | 1            | 0            | 0            | 5%                              |
| >35                            | 0            | 0            | 0            | 0%                              |
| Ethnicity                      |              |              |              |                                 |
| African                        |              |              |              |                                 |
| American                       | 2            | 0            | 1            | 15%                             |
| Hispanic                       | 0            | 2            | 5            | 35%                             |
| Caucasian                      | 5            | 3            | 2            | 50%                             |
| Asian                          | 0            | 0            | 0            | 0%                              |
| Other                          | 0            | 0            | 0            | 0%                              |

nt = not tabulated by Census Bureau
Note: percentages may not equal 100% due to rounding.
laboratory management.

• the perception of many frustrated ‘older’ techs.

The overwhelming majority (>85%) of the participants believed it would be important to build a strong bond between themselves and their job; “You must love what you do.” When asked if the CLS/CLT is an essential member of the healthcare team, the vast majority responded ‘yes’. Both groups believed they would have no difficulty finding a job once they had completed their education. In fact, many believed they would have jobs before they graduated. When asked if the field provided sufficient opportunities for growth the majority of the CLT/CLT participants (>75%) responded ‘no’. However, college biology students were more optimistic about advancement opportunities and expressed that they believed sufficient opportunities were in place.

Incentives to enter the CLS profession

Participants strongly supported the use of grant funding, scholarships, and loan forgiveness programs as means to attract more individuals to the CLS profession. Another suggestion was to “Show all of the ‘cool’ stuff you can do - use pictures” when recruiting. If students had to pick one incentive to attract candidates almost all (>85%) selected a grant program.

Questions regarding impressions about the stress of the job were asked. Generally, both groups were aware of the job stressors, but acknowledged this was a known element when they were making the decision to go or not to go into the field.

Participants were then asked to identify elements they looked for when selecting a professional career path. The greatest divergence of opinion between college biology juniors/seniors and CLS/CLT students was found with this question. The most frequently cited important job element for the biology students was the work environment, however for the CLS/CLT students the most frequently mentioned was salary (55%) followed by job satisfaction (33%). In addition the following were cited:

• Opportunities for advancement
• Flexible work hours
• Being part of the healthcare team

Finally, participants were asked to identify employer benefits that mattered most to them. They most frequently identified the following:

• Health insurance
• Paid vacation
• Retirement plan
• Tuition reimbursement

When participants were asked to identify one thing they believed would be most helpful in attracting students to a career in CLS, the most frequently cited by both groups was visibility at high school career fairs. Other suggestions included: money for education and a high school program to educate students about the wide variety of healthcare careers.

| Table 2. Characteristics of focus groups: junior/senior-level CLS/CLT students |
|---------------------------------|--------|--------|--------|--------|
| Gender                         | Houston (n=10) | Dallas (n=10) | Austin/San Antonio (n=15) | Texas (2006 US Census data) |
| Female                         | 8       | 6       | 13     | 77%    |
| Male                           | 2       | 4       | 3      | 33%    |
| Age (years)                    | 18-25   | 7       | 7      | 10     | 67%    |
|                                | 26-30   | 3       | 1      | 5      | 26%    |
|                                | 31-35   | 0       | 1      | 1      | 6%     |
|                                | >35     | 0       | 1      | 0      | 3%     |
| Ethnicity                      | African | 4       | 2      | 3      | 28%    | 18.0% |
|                                | American| 3       | 4      | 2      | 28%    | 35.1% |
|                                | Caucasian| 3      | 3      | 9      | 43%    | 49.5% |
|                                | Asian   | 0       | 0      | 2      | 6%     | 3.3%  |
|                                | Other   | 0       | 1      | 0      | 3%     | 2.0%  |

nt = not tabulated by Census Bureau
Note: percentages may not equal 100% due to rounding.
DISCUSSION

It was originally hypothesized that there would be more diversity of opinions between the college biology students and the CLS/CLT students, however, the results from the focus groups indicate that they hold many of the same attitudes and opinions. This may be due to fact that both groups are biology-oriented and enjoy working in the laboratory setting, thus leading to shared opinions on the points discussed during the focus groups sessions.

Several notable features emerge from these focus group discussions. They are:

1. Students see the CLS profession as an exciting career with diverse opportunities in which to practice the profession, however, the majority does not believe the salary is commensurate with the knowledge they must gain to enter into the profession.
2. Students who have rotated through a clinical laboratory observe that the CLS/CLT is being under-utilized, that some older laboratory workers are frustrated with their jobs, and that there is a lack of respect for the clinical laboratory worker.
3. A significant majority of students must work to pay for some or all of their education and would benefit from grants/scholarship programs or loan forgiveness programs.
4. The work environment and salary are important drivers for individuals who are considering or who are entering the field of practice.
5. While the job market is strong for CLS/CLT prepared individuals, many are looking for opportunities to advance which they do not readily see in the CLS/CLT profession.

Several key points of information can be gleaned from these focus group discussion results:

First, the CLS/CLT career is an attractive profession to healthcare oriented students, however, the profession continues to suffer from low visibility on college campuses, and perhaps even more important, in the healthcare arena.

Second, it is imperative for employers to develop suitable career paths for individuals who enter the CLS profession, as those entering now will soon be looking for the ‘next step’ in their career and this will only add to the personnel crisis the profession is experiencing. Employers would be well advised to develop a career ladder for the CLS/CLT and to look at what might be done to increase salaries for these essential members of the healthcare team. This point was also identified in the 2005 HSRA report on the CLS/CLT profession which found “the lack of a career ladder for laboratory professionals due to the relatively flat hierarchies that exist in hospital laboratories” is an issue in worker retention and recruitment.16

Third, the use of grants, scholarships and loan forgiveness programs hold promise as viable means to attract individuals to the CLS profession. This parallels the HRSA study which found that cost is a barrier for some students. “Several respondents cited the cost of education to students as a barrier to recruitment into clinical laboratory science programs.”16

These focus group studies highlighted information not previously reported regarding students and the clinical laboratory science profession.

• CLS/CLT students are concerned about what they are observing in clinical laboratories during their clinical rotations: specifically, frustrated workers and a lack of respect given the clinical laboratory workers by other healthcare workers, primarily physicians and nurses.
• Unlike previous reports17 which indicated that the younger generation is not as interested in salary as previous generations, results from this study would indicate that salary is still an important driver in career direction for the CLS/CLT.
• Perhaps the most disturbing finding is that many of the individuals who are entering into the profession are not looking to remain in the profession as their long term career.
• It is hoped that the findings from this study will contribute to the understanding the interests and values of CLS/CLT graduates when addressing the problem of recruitment and retention in the CLS/CLT professions.

Clin Lab Sci encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to david.mcglasson@lackland.af.mil. In the subject line, please type “CLIN LAB SCI 22(1) RR MCCLURE”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

REFERENCES

3. Strategic laboratory workforce planning—you cannot afford not to do


A Poisson-based Prediction Model and Warning System for MRSA Daily Burden

ROCCO J PERLA, BRADFORD D ALLEN

OBJECTIVE: This study was designed to demonstrate that the number of methicillin-resistant Staphylococcus aureus (MRSA) isolates collected daily in a community hospital is Poisson distributed and that using a one-sided Poisson control table is a fast and easy way to recognize unusually high numbers of MRSA isolates collected daily that may signal possible outbreaks.

METHODS: A retrospective analysis of MRSA isolates collected daily over a three year period (2005-2007, N = 934) was performed. Observed MRSA isolate frequencies are compared to Poisson frequencies using chi-square goodness-of-fit tests. A regression equation on the mean number of MRSA isolates collected daily for the years 2005, 2006, and 2007 is used to predict the mean number of MRSA isolates for 2008. A warning system for MRSA isolates collected daily is presented and a one-tailed, mean + 2 sigma control table is provided.

SETTING: One-hundred-fifty bed community hospital in central Massachusetts.

RESULTS: Goodness-of-fit tests showed close agreement between actual MRSA isolates collected daily and Poisson frequencies for 2005 ($\chi^2_4 = 4.045, p = 0.39$), 2006 ($\chi^2_4 = 2.807, p = 0.59$), and 2007 ($\chi^2_4 = 1.494, p = 0.83$).

CONCLUSION: Theoretical and empirical support is provided for the Poisson probability model. The model can be used to identify unusually high occurrences of MRSA isolates collected daily. This study was limited to a single community healthcare system but the results may be generalized to other types of healthcare settings.

ABBREVIATIONS: ICPs = infection control practitioners; MDROs = multi-drug resistant organisms; MRSA = methicillin-resistant Staphylococcus aureus.

INDEX TERMS: infection control; microbiology; MRSA; Poisson distribution; statistical process control.

as to when epidemiologic follow-up is reasonable. To date, the number of MRSA isolates (or other MDROs) collected daily in a hospital setting have not been modeled with the Poisson distribution.

MATERIALS AND METHODS

Data collection

The statistics presented here provide a retrospective analysis of the number of MRSA isolates collected daily over a three year period (2005-2007) at a 150-bed, non-major teaching community hospital in central Massachusetts. The range of services provided by the testing laboratory was consistent over the three year period and included inpatient and outpatient services. Laboratory records of MRSA isolates recovered daily during the years 2005 to 2007 were collected using the Vitek DataTrac Logbook Report program (bioMerieux, Durham NC) following the “first isolate rule” (i.e., one patient isolate per year) and following Clinical Laboratory and Standards Institute guidelines for the analysis of susceptibility data. The MRSA isolate data were grouped into daily occurrence categories (0 to ≥5 occurrences per day) for each of the three years. The grouping procedure was repeated on two separate occasions to insure reliability. The data included all clinical isolates (inpatients and outpatients), with duplicate patient isolates, surveillance cultures, and screens omitted from the analysis. The actual specimen collection date (not the date reported to infection control) was used to organize the data because the date of collection provides a reasonable and standard estimate of frequency across time. When the warning system presented here is used in real time, MRSA counts by date of collection should be used rather than counts by date reported to infection control so false warnings are not generated by batched reports.

Statistical analysis

To conclude that MRSA isolates collected daily are Poisson distributed, it was assumed that the occurrence or non-occurrence of MRSA isolates in any small time interval is a Bernoulli trial (an experiment with two possible outcomes e.g., yes/no or infection/no infection). It was then observed that the mean number of MRSA isolates collected daily was independent of time interval length or period (i.e., seasonally, monthly, weekly, daily). To test whether the observed frequencies of MRSA isolates collected daily were Poisson distributed, the number of MRSA isolates collected daily during the years 2005 to 2007 were compared to Poisson frequencies using chi-square goodness-of-fit tests. Poisson frequencies were computed using $e_i = np$, where $n$ is the total number of MRSA isolates and $p_i$ is the Poisson probability of $i$ occurrences where $i$ is 0, 1, 2, 3, 4, ≥5. Goodness-of-fit

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of MRSA isolates collected daily</th>
<th>Total</th>
<th>$\lambda^*$</th>
<th>$s^2$</th>
<th>Skew</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>≥5</td>
</tr>
<tr>
<td>2005</td>
<td>210</td>
<td>105</td>
<td>39</td>
<td>9</td>
<td>2</td>
<td>0</td>
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<tr>
<td>%</td>
<td>57.5</td>
<td>28.8</td>
<td>10.7</td>
<td>2.5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td>153</td>
<td>119</td>
<td>68</td>
<td>21</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>42.0</td>
<td>32.6</td>
<td>18.6</td>
<td>5.7</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>2007</td>
<td>132</td>
<td>131</td>
<td>65</td>
<td>30</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>%</td>
<td>36.2</td>
<td>35.9</td>
<td>17.8</td>
<td>8.2</td>
<td>1.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*lambda (mean of the Poisson distribution)
†variance (≈$\lambda$)
‡Skew (≈$1/\sqrt{\lambda}$)
§coefficient of variation

Note: categories 4 and ≥5 were combined for $\chi^2$ analysis.
tests were used for each of the three years. To follow common goodness-of-fit test guidelines, categories with ≥4 occurrences of MRSA isolates per day for the years 2006 and 2007 were combined to ensure that no more than 20% of the expected frequencies were less than 5. After all tests of the Poisson model were confirmed (see the Results section), the Poisson distribution was used to develop a MRSA daily burden warning system. The mean, variance, skew, and coefficient of variation (variance/mean) of MRSA isolates collected per day were found and compared to the theoretical Poisson distribution for each of the three years. It should be noted that for the Poisson distribution, the mean and variance are equal. That is, the mean = λ (lowercase Greek letter lambda), variance = λ, coefficient of variation = 1, and skew = 1/√λ.

Finally, a regression equation for the mean based on the years 2005, 2006, and 2007 was found and used to predict the mean number of MRSA isolates for 2008.

RESULTS

Distribution analysis

Goodness-of-fit tests showed close agreement between the number of MRSA isolates collected daily and Poisson frequencies for 2005 (χ² = 4.045, p = 0.39), 2006 (χ² = 2.807, p = 0.59), and 2007 (χ² = 1.494, p = 0.83). Close agreements in variance and skew were found between the Poisson distribution and the actual numbers of MRSA isolates collected daily for each of the three years (Table 1).

Prediction and warning limits

The mean of a Poisson distribution is the only parameter necessary to completely describe the Poisson distribution. To predict the mean number of MRSA isolates per day in 2008, a regression equation of the form λₚ = a + bt was found and used to describe the linear trend of the mean during 2005, 2006, and 2007. The coefficient of determination of the equation was found to be R²=0.93. The regression equation was used to predict the mean value (and thus the distribution) for 2008. Using the regression equation, the mean number of MRSA isolates collected daily is predicted to be λₚ = 1.29 (95% CI, 1 – 1.58). (The value of λₚ = 1.29 is very close to the predicted mean value of our longer-term logistic model for the years 2003 - 2007. The logistic model is not presented here). The predicted value of lambda for 2008 (λₚ = 1.29) can be used to construct a table that serves as a warning system for unusually high numbers of MRSA isolates collected per day. Isolate occurrences that exceed the “follow-up recommended” values presented in Table 2 would trigger epidemiologic follow-up. Table 2 presents a range of lambda (mean) values, lambda plus 2-sigma values, the numbers of occurrences where follow-up is recommended (≥ lambda plus 2-sigma), and the probabilities of a false alarm. Although it is standard practice to use 3-sigma warning limits in statistical process control models, a more cautious 2-sigma warning limit is used here that is more sensitive to potential infection control problems (but is more likely to have false alarms). For example, using a 3-sigma model as a warning limit would produce false alarms only once in 769 days (1/0.0013) on average, but would probably miss all the real warning signals. Of course, the follow-up warning limits can be adjusted in different situations where it may be more prudent to reduce the risk of false alarms.

<table>
<thead>
<tr>
<th>λ (mean)</th>
<th>λ + 2 (sigma)*</th>
<th>X ≥ λ + 2 (sigma)</th>
<th>Probability X ≥ λ + 2 (sigma)</th>
<th>Follow-up X ≥ λ + 2 (sigma)</th>
<th>Probability of a false alarm</th>
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</thead>
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<tr>
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<td>0.08</td>
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<tr>
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<td>X ≥ 4</td>
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<td>3.77</td>
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<td>1.75</td>
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<td>4.83</td>
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</table>

*sigma is equal to the square root of lambda (√λ)
DISCUSSION
The need to address aspects of infectious disease epidemiology, such as the use of more systematic, rigorous and logical methods of information management, has been advocated by many, particularly in the context of statistical process control and signal detection theory. Today, the increased frequency and emergence of resistant microorganisms and other public health threats necessitate the use of more formal decision-making systems that augment the clinical interpretative dimension of laboratory and infection control practice. It has long been recognized that the microbiology laboratory is an “early warning center” for potential infection control problems and that microbiologists and ICPs need to work together to develop optimal surveillance strategies. There are some aspects of clinical microbiology and infection control practice that are ideally suited for probabilistic modeling and surveillance, including frequency, distribution, and time series analysis of specified microorganisms. However, many microbiologists and ICPs may not be familiar or comfortable with probability modeling, thereby limiting the use of these important tools. This study demonstrates that the frequency of MRSA isolates collected daily in a community hospital is Poisson distributed and that a Poisson model can be used to determine when isolate frequencies per day indicate the need for a higher level of attention or action while understanding the chance of acting on a false alarm. Although the Poisson model presented here focuses on MRSA, the model is also useful for monitoring other MDROs such as extended-spectrum β-lactamase producing gram negatives, or vancomycin-resistant enterococcus.

The model presented here is appropriate, easy to use, and accessible to a large number of microbiologists and ICPs who may not have advanced training in statistics. Further, the model may be implemented using control chart features for modeling Poisson data that are available in many statistical packages such as Stata (Stat Corporation, College Station TX) and SPSS (SPSS Inc. Chicago IL). In addition, the probability model also provides a means of correcting misperceptions in clinical judgment and intuition. For example, prior to beginning this study, hospital infection control and microbiology staff members were asked about the expected frequency of zero MRSA isolate days during 2007. All three ICPs and seven microbiologists believed there would be few or no days with no MRSA isolates collected. Their expectations were incorrect however because finding no MRSA isolates daily was the most frequent category in each of the three years studied (2005-2007). In this case, the infection control and microbiology staff thought the MRSA daily occurrence was far greater than it actually was.

Reducing MRSA and other MDRO frequencies over time is of preeminent importance and probability modeling needs to be on the front lines of this effort. If MRSA frequency is reduced in a defined area (e.g., community, hospital, outpatient service), then the change would be reflected in the distribution model and related statistics (mean, variance, skew and Poisson probabilities). In situations where reduction in frequencies is subtle, the warning system cutoff points can help quantify the effectiveness of different MDRO reduction efforts. Because infection control is becoming a more interdisciplinary and multidisciplinary activity, particularly in the area of antibiotic resistance surveillance, it is likely that probability models will become important infection control tools that allow, in the spirit of W.E. Deming, the sound use of probability and statistics as a basis for action.

REFERENCES
A Long-term Forecast of MRSA Daily Burden Using Logistic Modeling

BRADFORD D ALLEN, ROCCO J PERLA

OBJECTIVE: This article presents a logistic model that describes the mean number of unique methicillin-resistant Staphylococcus aureus (MRSA) isolates collected daily at a 150-bed community hospital in central Massachusetts. The model is used to derive a long-term forecast of the mean MRSA isolate frequency.

METHODS: The mean number of MRSA isolates collected daily was found for each quarter from the first quarter of 1996 to the first quarter of 2008. A logistic model was fit to the data and then extrapolated to obtain a long-term forecast.

SETTING: Data was collected at a one-hundred-fifty bed community hospital in central Massachusetts.

RESULTS: The coefficient of determination indicates that 87% of the variation in transformed data is explained by the model. The extrapolated logistic model prediction is that the mean number of MRSA isolates collected daily approaches 1.42 MRSA isolates per day.

CONCLUSION: Logistic modeling of empirical data using modest mathematical assumptions is an effective way to understand, visualize, and forecast MRSA daily frequencies over time. The advantage for laboratorians and epidemiologists is that logistic models provide reliable trending and long-term prediction ability of multi-drug resistant organism frequencies. Moreover, as additional data is obtained, the logistic model assumptions can be checked, the model updated, and forecasts improved.

ABBREVIATIONS: MRSA = methicillin-resistant Staphylococcus aureus.

INDEX TERMS: infection control; logistic model; microbiology; MRSA; Poisson distribution.


Bradford D Allen EdD is chair, Department of Mathematics and Science, Lasell College, Newton MA.

Rocco J Perla EdD is IHI/George W Merck Fellow, Institute for Healthcare Improvement, Cambridge MA; and section head, Clinical Microbiology & Diagnostic Immunology, and epidemiologist, Department of Infection Control and Prevention, HealthAlliance Hospital, Leominster MA.

Address for correspondence: Bradford D Allen EdD, chair, Department of Mathematics and Science, Lasell College, 1844 Commonwealth Avenue, Newton, MA 02466. (978) 443-1815. ballen@lasell.edu.

ACKNOWLEDGEMENT: The authors thank Zachary M Callahan for his valuable assistance organizing the multiple data sets used in the analysis.

This article presents a long-term forecast of the mean daily frequencies of unique methicillin-resistant Staphylococcus aureus (MRSA) isolates. The forecast is found by extrapolating a logistic model that is derived from MRSA isolate data collected daily at a 150-bed community hospital in central Massachusetts from the first quarter of 1996 through the first quarter of 2008. The logistic model used to generate the forecast was first described by the Dutch mathematical biologist PF Verhulst in 1838 and later by R Perl in 1920. The model has good predictive ability provided its moderate assumptions remain approximately true. In addition to providing a reasonable forecast of MRSA burden, the research presented here shows the value of using historical laboratory data to describe important laboratory processes and identify trends. In another article appearing in this journal, we present theoretical and empirical support for a Poisson distribution model of MRSA isolates collected daily at the same central Massachusetts hospital. The Poisson probability model provides a reliable way to recognize when unusually high numbers of MRSA isolates may signal
MRSA outbreaks. If the Poisson model of MRSA isolates is combined with the logistic model presented here, then the forecast may be seen as a prediction of the long-term mean of Poisson-distributed MRSA isolates collected daily. The prediction is based on over twelve years of data collected at the same Massachusetts hospital. If the models are treated separately, as is done in the two articles appearing in this issue, the Poisson model provides a statistical warning system that signals when high MRSA frequencies may signal an outbreak. Alternatively, the logistic model provides a deterministic trend and long-term forecast of MRSA daily burden. Moreover, these models could be used to monitor and analyze other multi-drug-resistant organism frequencies over time.

MATERIALS AND METHODS
Laboratory records of MRSA isolates recovered daily from the first quarter of 1996 through the first quarter of 2008 were collected using the Vitek DataTrac Logbook Report program (bioMerieux, Durham NC) following the “first isolate rule” (i.e., one patient isolate per year) and following Clinical and Laboratory Standards Institute guidelines for the analysis of susceptibility data. The data include all clinical isolates (inpatients and outpatients), with duplicate patient isolates, surveillance cultures, and screens omitted from the analysis. The actual specimen collection date (not the date reported to infection control) was used to organize the data because the date of collection provides a reasonable and standard estimate of frequency across time. The mean number of MRSA isolates collected daily was found for each quarter over a 12.25 year time interval. The means were smoothed using a four-quarter moving average and changes in the mean number of MRSA isolates from one quarter to the next were found.

RESULTS
A plot of the mean number of MRSA isolates collected daily for each quarter is shown in Figure 1. After the MRSA means were smoothed using a four-quarter moving average and changes in the means were found, the changes were plotted in terms of the size of the means. Figure 2 shows the relationship between changes in the mean number of MRSA isolates collected daily and the actual mean number of isolates. The plot shows that as the mean number of isolates increases, the change in the number of isolates collected daily increases at first but then declines. It can be seen from Figure 2 that the change in the number of isolates per day from quarter to quarter reaches a maximum of 0.09 isolates when the mean number of isolates per day is 0.68. This observed maximum occurs in the 40th quarter and we expect that the true maximum, $M$, also occurs around this time.

If the change in the mean number of MRSA isolates collected daily from quarter to quarter continues its decline toward zero, then growth in the mean number of MRSA isolates collected daily will begin to level off, and over the long-term, remain at a stable level close to the value $M$. Drug-resistant organisms frequently persist in environments at stable levels as the organisms become more established. Stable organism levels imply that changes in the levels have declined to zero. Figure 2 shows a pattern where the rate of change is beginning to decline. Such a pattern would occur if the change in
the number of isolates collected daily and the actual number of isolates collected daily are proportional, but the rate of proportionality is declining. If the change in the number of isolates approaches zero as the actual number of isolates approaches \( M \), then as Figure 2 suggests, the change in the mean number of isolates can be approximated with a quadratic function. If we let \( S \) represent the mean number of MRSA isolates collected daily and let \( M \) represent the point at which the rate of change reaches zero, we can express the change in the mean number of isolates in terms of the actual mean number of isolates using a quadratic equation with zeros at \( S=0 \) and \( S=M \). That is:

\[
\frac{dS}{dt} = r(M - S)S \tag{1}
\]

where \( r > 0 \) is a constant that reflects the maximum possible rate of change in the mean number of isolates. Using separation of variables and partial fractions, we find that:

\[
\ln\left(\frac{S}{M - S}\right) = rMt + C \tag{2}
\]

The right-hand-side of Equation 2 shows that \( \ln\left(\frac{S}{M - S}\right) \) is linear in terms of \( t \), where \( t \) is the number of quarters starting from the first quarter of 1996. Using Figure 2, we estimate that \( M \approx 1.4 \). To test this model further, we use the actual data values plotted in Figure 1 for values of \( S \) and plot \( \ln\left(\frac{S}{M - S}\right) \) in terms of \( t \) from 1996 to 2008. If the model is good, the resulting plot will be linear in terms of \( t \). Figure 3 is a plot of the transformed data \( \ln\left(\frac{S}{M - S}\right) \) in terms of \( t \). To get the best linear relationship, the value of \( M \) was adjusted to \( M = 1.42 \). The resulting coefficient of determination for the linear model reveals that 87% of the variation in the transformed data is explained by the linear model. Solving Equation 2 for \( S \) gives a logistic model whose graph is shown in Figure 4.

To see the long-term trend of the mean number of MRSA isolates collected daily, the logistic model is extended several more years in Figure 5. The logistic model shows that the mean number of MRSA isolates collected daily approaches a stable long-term level of \( M = 1.42 \) MRSA isolates per day.

DISCUSSION

Clinical laboratory scientists, researchers, microbiologists, and epidemiologists would benefit from using MRSA daily frequency data and logistic modeling to make informed decisions about MRSA trends. For example, the logistic model can be used to predict MRSA frequencies well before
the long-term frequency is reached. Researchers should be aware, however, that growth in a restrictive environment can approach a stable level in different ways. As our data begins to show, the number of isolates can level off by means of a gradual decrease in the rate of change. Alternatively, the mean isolate frequency can move past a long-term level but might then decrease in a smooth or an oscillatory way. Long-term stability also occurs if isolate frequency exceeds a potential long-term stable level but by doing so, new restrictions on growth and transmission that reduce the long-term frequency are generated. For example, an outbreak could focus intense and persistent infection control measures that result in a long-term endemic state at a lower level than had the outbreak not occurred.

Logistic growth models can give insights into claims by researchers and infection control professionals where significant treatment or intervention effects were observed on experimental or observational studies or in reports from the field. Limitations to growth in the environments of drug-resistant organisms may lead some to incorrectly attribute a reduction in MRSA growth and transmission to an intervention program or initiative when the reduction may be nothing more than a reflection of the natural evolution of the organisms as described by the logistic model. At a minimum, the effectiveness of reduction efforts directed at MRSA and other multidrug resistant organisms (MDROs) needs to be measured and assessed in the context of the natural evolution of these organisms. Clinical laboratory scientists, epidemiologists, healthcare quality professionals, and manufacturers of susceptibility testing systems should recognize the importance of using time-series data and logistic modeling to develop trending and forecasting models for MDROs.

Clin Lab Sci encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to david.mcglasson@lackland.af.mil. In the subject line, please type “CLIN LAB SCI 22(1) RR ALLEN”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

REFERENCES
An Evaluation of the AdvanDx Staphylococcus aureus/CNS PNA FISH™ Assay

DONNA M HENSLEY, RACHEL TAPIA, YADIRA ENCINA

PURPOSE: A study was conducted to compare the *S. aureus*/CNS PNA FISH™ Culture Identification Kit (AdvanDx, Woburn MA) to standard microbiology identification methods for presumptive identification of *S. aureus* and coagulase-negative staphylococcus (CNS) in positive blood cultures.

MATERIALS AND METHODS: Blood cultures (n=301) that signaled positive on the BacT/Alert™ 3D (bioMerieux, Durham NC) automated blood culture system and had gram-positive cocci in clusters on Gram stain were processed using standard microbiology methods and were analyzed with the *S. aureus*/CNS PNA FISH™ assay. The *S. aureus*/CNS PNA FISH™ assay was performed in accordance with the manufacturer’s instructions.

RESULTS: Overall agreement was 96.7%. Sensitivity for *S. aureus* was 96.5% (83/86). Specificity for *S. aureus* was 100% (215/215). Sensitivity for CNS was 96.6% (201/208). Specificity for CNS was 96.8% (90/93). Three cultures reported as *S. aureus* were identified as CNS by *S. aureus*/CNS PNA FISH™. Three cultures that reported only *S. aureus* were positive for both *S. aureus* and CNS by *S. aureus*/CNS PNA FISH™. One *S. viridans* was identified as CNS by *S. aureus*/CNS PNA FISH™. Seven cultures that reported CNS were negative by *S. aureus*/CNS PNA FISH™.

CONCLUSION: When used in conjunction with Gram stain and culture the *S. aureus*/CNS PNA FISH™ assay may be beneficial in terms of reducing time to correct therapy, thereby decreasing mortality and costs associated with *S. aureus* bacteremia.

ABBREVIATIONS: CNS = coagulase negative staphylococci; FISH = fluorescent in situ hybridization; PNA = peptide nucleic acid.

INDEX TERMS: blood culture; PNA FISH; *S. aureus*.

The peer-reviewed Research and Reports Section seeks to publish reports of original research related to the clinical laboratory or one or more subspecialties, as well as information on important clinical laboratory-related topics such as technological, clinical, and experimental advances and innovations. Literature reviews are also included. Direct all inquiries to David L McGlasson MS CLS(NCA), 59th Clinical Research Division/SGRL, 2200 Bergquist Dr., Bldg 4430, Lackland AFB TX 78236-9908, david.mcglasson@lackland.af.mil

ACKNOWLEDGEMENTS: This research was performed under the authority of the Department of Defense and the 59th Medical Wing, 59th Clinical Research Division, Lackland AFB TX Institutional Review Board. It was a poster presentation at the Society of Armed Forces Medical Laboratory Scientists (SAFMLS) Annual Meeting, New Orleans LA, March 10-14 2008.

DISCLAIMER: This material represents the personal statements of the authors and is not intended to constitute an endorsement by the 59th Medical Wing or any other federal entity.

Septicemia is one of the leading causes of death in the United States. *Staphylococcus aureus* infections, including *S. aureus* septicemia, place economic and personal burdens on the healthcare system in terms of increased morbidity, mortality, length of hospital stay, and associated costs. While coagulase negative staphylococcus (CNS) is the organism most commonly isolated from positive blood cultures it is often a contaminant. Both *S. aureus* and CNS are seen as gram-positive cocci in clusters (GPCC) on a Gram stained smear and this is typically the first report that a clinician receives regarding a positive blood culture. Differentiation of contamination from true bacteremia is important in
terms of treatment and there are differing opinions, even among infectious diseases experts, about the best strategy to follow.\textsuperscript{10-13} Equally important is the differentiation of \textit{S. aureus} and CNS in cases of true bacteremia. Concerns about the overuse or misuse of vancomycin must be weighed against the need for proper antimicrobial treatment of methicillin-resistant \textit{S. aureus} (MRSA) bacteremia. Information supplied by the clinical microbiology laboratory to clinicians plays an important role in decision making. Traditional microbiology methods of identification involving subculture to solid media and biochemical testing take one to two days for an identification of \textit{S. aureus} to be confirmed during which time the decision must be made whether or not to start empiric therapy. Evaluation of rapid methods for identification of \textit{S. aureus} directly from blood culture bottles, including real-time polymerase chain reaction (PCR), peptide nucleic acid fluorescent in situ hybridization (PNA FISH), PNA FISH combined with flow cytometry, tube coagulase, and the API RAPID DEC staph system (bioMerieux, Durham NC), have been reported in the literature.\textsuperscript{14-18} Rapid testing and providing a presumptive identification to the clinician within hours of positivity have the potential to improve management of \textit{S. aureus} bacteremia by decreasing the time to appropriate therapy.\textsuperscript{19} \textit{S. aureus}/CNS PNA FISH™ (AdvantDX, Woburn MA) is a FISH method that utilizes PNA probes specific for \textit{S. aureus} and non-aureus 16S ribosomal RNA sequences. The test is performed on smears made directly from positive blood culture bottles and results are available in approximately three hours. The inclusion of probes for both \textit{S. aureus} and CNS allows not only differentiation of the two organisms but also serves as a backup for Gram stain interpretation since nonstaphylococcal isolates which may be misinterpreted as GPCC will not stain with the \textit{S. aureus}/CNS PNA FISH™ probes. This study evaluated the performance of the \textit{S. aureus}/CNS PNA FISH™ assay when compared to traditional microbiology methods of identification.

**METHODS**

Blood cultures collected as standard of care and submitted to two clinical microbiology laboratories were incubated on BacT/Alert™ 3D (bioMerieux, Durham NC) automated blood culture systems. Blood culture bottle types were BacT/Alert™ PF, BacT/Alert™ SA 40 mL, and BacT/Alert™ SN 40 mL. Cultures that signaled positive on the BacT/Alert™ 3D were Gram stained and were included in the study if the Gram stain was reported as GPCC (n=294). Several blood cultures positive with non-staphylococcal isolates (n=7) were included in the study to evaluate the specificity of the \textit{S. aureus}/CNS PNA FISH™ assay. Isolates were identified by standard microbiology techniques. Briefly, broth from positive bottles was subcultured to trypticase soy agar with 5% sheep’s blood (TSA II, BBL, Becton-Dickinson and Company, Sparks MD) and the plates were incubated at 35±1°C in 5% CO\textsubscript{2}. Following overnight incubation a catalase test (hydrogen peroxide, Brite-Lite, AmerisourceBergen, Valley Forge PA) and a Staphaurex (Remel, Lenexa KS) test were performed on all isolates exhibiting typical staphylococcal morphology. Isolates that were catalase positive and Staphaurex positive were identified as \textit{S. aureus}. Isolates that were catalase positive and Staphaurex negative were identified as CNS. Each positive bottle was also analyzed by \textit{S. aureus}/CNS PNA FISH™ for presumptive identification of \textit{S. aureus} or CNS. The \textit{S. aureus}/CNS PNA FISH™ slides were batched and transported to a separate research lab for analysis. The manufacturer's instructions for the \textit{S. aureus}/CNS PNA FISH™ assay were followed. Briefly, a drop of positive blood culture broth was mixed with a drop of fixation solution on the microscope slide provided with the kit. The slides were air dried and then fixed by dipping them in methanol for three to five seconds and allowing them to air dry again. The slides were then immersed in 80% ethanol for 10 minutes and air dried again. One drop of \textit{S. aureus}/CNS PNA hybridization solution (PNA reagent), containing a mixture of a fluorescein-labeled \textit{S. aureus} specific PNA probe and a rhodamine-labeled PNA probe specific for non-aureus staphylococci, was added to the smear after which the slides were coverslipped and incubated at 55 ± 1°C for 90 ± 5 minutes in the PNA FISH Workstation (AdvantDx). The slides were immersed in preheated (55 ± 1°C) wash solution, gently agitated to remove the coverslips, and then incubated in the wash solution at 55 ± 1°C for 30 ± 5 minutes. The slides were air dried and then one drop of mounting media and a coverslip were added to each slide. The slides were examined for fluorescence within two hours using a Nikon Optiphot microscope with an EXFO X-Cite 120 Fluorescence Illumination System (Photonic Solutions, Inc, Mississauga Ontario, Canada) and a 100X oil objective. The microscope was equipped with a FITC/Texas Red dual band filter (AdvantDx, Woburn MA). \textit{S. aureus} appeared as green fluorescence, CNS appeared as red fluorescence, and nonstaphylococcus isolates did not fluoresce. A positive control slide containing both \textit{S. aureus} and CNS and a negative control slide were included on each run. The identifications obtained by the standard microbiology methods and the \textit{S. aureus}/CNS PNA FISH™ assay were compared.
RESOLUTION
Overall agreement between the two methods was 96.7% (291/301). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for S. aureus and CNS. See Table 1. Three isolates identified as S. aureus by standard testing methods were identified as CNS by S. aureus/CNS PNA FISH™. Seven cultures that were reported as positive for CNS using the standard testing methods for identification were negative by S. aureus/CNS PNA FISH™. For three cultures in the study S. aureus was the only isolate reported following standard testing methods but both S. aureus and CNS were seen on the S. aureus/CNS PNA FISH™ smears. Isolates identified as Enterococcus faecalis, Group B Streptococcus, Micrococcus sp., and Candida albicans by standard testing methods were negative when analyzed by the S. aureus/CNS PNA FISH™ assay. There were two cultures included in the study that were identified as Streptococcus viridans by standard testing methods. One of the S. viridans cultures was negative by S. aureus/CNS PNA FISH™ and the other was identified as CNS by S. aureus/CNS PNA FISH™.

DISCUSSION
Historically, and in contrast to other sections of the clinical laboratory, there have been few “stat” procedures in the microbiology laboratory. That situation is changing due to the advancement of molecular techniques and the availability of molecular diagnostic assays. Having the ability to provide rapid accurate diagnostic information to clinicians is a goal of most clinical microbiology laboratories. The S. aureus/CNS PNA FISH™ assay is available on the market in kit format and enables the microbiology laboratory to provide rapid presumptive identification of S. aureus and CNS. With the exception of methanol and 80% ethanol all reagents needed to complete the assay are included in the kit so reagent preparation time, including dilution of the wash buffer, is minimal. Slide preparation was easily integrated into our normal routine for handling positive blood culture bottles. We found it best to prepare and read the Gram stain and then prepare the PNA/FISH slide if necessary. However, AdvanDx is marketing PNA/FISH assays for other organisms and, with the exception of the organism specific PNA reagent, all kit components are interchangeable. Currently kits for E. faecalis/Other Enterococcus and C. albicans/C. glabrata are available. As more kits are introduced it may become more time effective to prepare the Gram stain and PNA FISH slides at the same time and then select the correct PNA FISH assay to perform based on the Gram stain result. Even though slide preparation is easily integrated into the daily routine the S. aureus/CNS PNA FISH™ assay is not an “on demand” test like the Gram stain. The actual hands-on time to perform the S. aureus/CNS PNA FISH™ assay is approximately 12-15 minutes. But because of the drying and incubation periods the total time from slide preparation to reading for fluorescence is approximately three hours. For practical reasons slides need to be batched, thereby eliminating the PNA FISH assay from a true “stat” procedure. Still, the decrease in time from 24 hours to 3 hours from time of positivity to presumptive identification could have a significant impact on patient care. The PNA FISH Workstation will accommodate up to 30 slides for the 90 minute incubation. Depending on the laboratory workload one or more batches of slides could be assayed during the day.

The S. aureus green fluorescence was very intense and easily read on every S. aureus/CNS PNA FISH™ slide that was positive for S. aureus. The red fluorescence for the CNS was variable in our study, ranging from a vivid red that was easily distinguished to a dull red that could barely be differentiated from the reddish background. Perhaps the fluorescence variability contributed to the fact that we had seven CNS culture positive specimens that were S. aureus/CNS PNA FISH™ negative. It is possible that the cells were there but the fluorescence could not be seen. The poor fluorescence was not confined to single runs and did not correlate with the number of cells present in the smear or a particular type of blood culture bottle. We made certain that the entire surface of the blood smear

<table>
<thead>
<tr>
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<th>S. aureus</th>
<th>CNS</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>96.5% (83/86)</td>
<td>96.6% (201/208)</td>
</tr>
<tr>
<td>Specificity</td>
<td>100% (215/215)</td>
<td>96.8% (90/93)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100% (83/83)</td>
<td>98.6% (201/204)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98.5% (215/218)</td>
<td>92.8% (90/97)</td>
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was covered with the PNA reagent and that bubbles were not present under the coverslip during the hybridization period so we feel confident that each slide had an equal exposure to the PNA reagent. One shortcoming of this study is that not all of the CNS isolates were identified to species level. If we had this information we may have been able to correlate the poor staining with certain species of CNS. However, in this study, we could not identify a factor that contributed to the poor fluorescence seen on some of the CNS slides.

Unfortunately we were unable to resolve the ten discrepancies in this comparison study. None of the blood culture bottles and only one of the isolates were available for retesting. All ten of the *S. aureus*/CNS PNA FISH™ slides were reexamined and the reported results were confirmed. The one available isolate was retested and confirmed as *S. aureus*. Since the corresponding *S. aureus*/CNS PNA FISH™ slide for this isolate was reexamined and confirmed as CNS the discrepancy remains unresolved.

Overall we found the *S. aureus*/CNS PNA FISH™ assay easy to perform and interpret. The inclusion of the assay in the routine workup of positive blood cultures that have GPPC on Gram stain could provide information to clinicians that would be useful in determining appropriate antimicrobial therapy thereby decreasing morbidity, mortality, length of hospital stay, and associated costs. However, the fact that we had three *S. aureus* positive cultures that were identified as CNS by *S. aureus*/CNS PNA FISH™ emphasizes that the *S. aureus*/CNS PNA FISH™ assay should be used as an adjunct to traditional microbiology testing methods and not replace them.

*Clin Lab Sci* encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to david.mcglasson@lackland.af.mil. In the subject line, please type “*CLIN LAB SCI 22(1) RR HENSLEY*. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

REFERENCES
RESEARCH AND REPORTS

Methicillin-resistant *Staphylococcus aureus* Fomite Survival

CHRISTA WILLIAMS, DIANE L DAVIS

OBJECTIVE: To assess survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on fomites encountered by health students.

DESIGN: Three suspensions of MRSA were made to mimic lab splashes: a 0.5 McFarland trypticase soy broth, whole blood with 50 colony forming units/mL, and body fluid/serum with 2,000 colony forming units/mL. These were seeded onto three environmental surfaces (glass, vinyl floor tile, and countertop) and wet swabbed for 60 days. High touch areas of student stethoscopes were also wet swabbed. MRSA selective CHROMagar® was used to identify organism survival.

SETTING: Salisbury University, Salisbury MD

PARTICIPANTS: Salisbury University nursing and respiratory therapy students who volunteered to have their stethoscopes swabbed anonymously.

MAIN OUTCOME MEASURE: Detection of pink colonies on MRSA-selective CHROMagar®.

RESULTS: MRSA in 0.5 McFarland broth lived for >60 days on all surfaces. MRSA in blood was undetectable on any surface, and MRSA in serum survived 41 days on glass, 45 days on tile, and >60 days on countertop. Five of thirty-three stethoscopes (15%) tested were positive for MRSA.

CONCLUSIONS: Previous studies showed fomite survival of MRSA for about two weeks using contact plate sampling and MRSA on 7.4% of stethoscopes. We showed longer MRSA survival times by wet swab sampling and a higher stethoscope contamination rate. As expected, higher organism loads survived longer.

ABBREVIATIONS: ATCC = American Type Colony Collection; CFUs = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*; TS = trypticase soy.

INDEX TERMS: fomite; methicillin-resistant *Staphylococcus aureus*; MRSA; survival.

**Clin Lab Sci 2009;22(1):34**

Christa Williams MT (ASCP) and Professor Diane L Davis PhD CLS(NCA) MT SC SLS (ASCP) are of the Clinical Laboratory Science Program, Health Sciences Department, Salisbury University, Salisbury MD.

Address for correspondence: Diane L Davis PhD CLS(NCA), professor, Clinical Laboratory Science Program, Health Sciences Department, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801. (410) 548-4787, (410) 548-9185 fax. dldavis@salisbury.edu

ACKNOWLEDGEMENTS: This research was presented in July 2008 at the American Society of Clinical Laboratory Science Student Poster Contest in Washington DC. Grant funding for laboratory supplies came from the Salisbury University Student Academic Research Awards Fund and the Salisbury University Henson School of Science and Technology Student Research Award Fund.

Multi-drug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) are significant causes of infection in the healthcare environment and increasingly in the community.1 The escalating antibiotic resistance of this virulent nosocomial pathogen makes MRSA infections especially difficult to treat.2 Since MRSA can harbor on many surfaces in hospitals such as floors and door handles even after disinfection,3 MRSA can be indirectly transmitted through fomites, or contaminated inanimate objects, causing infections.1,3 One study showed that the rate of transfer of *Staphylococcus aureus* organisms from a contaminated object from a single hand contact was 100%, so the degree of risk from fomites is relatively high.4 Accurate knowledge is therefore needed regarding how long the organism survives, and this can be accomplished by seeding various surfaces with a known isolate of MRSA and monitoring for a period of time. The growth medium CHROMagar® selectively inhibits many organisms and discriminates methicillin-sensitive staphylococci (MSSA) from MRSA.5 This allows rapid identification of MRSA from non-living environmental samples.

There is extensive literature on survival of organisms on inanimate objects, but since the number of potential objects and
organism loads is almost infinite, the literature is necessarily incomplete. Further, a comprehensive review in 2006 noted that the data from many of the studies is contradictory. For example, one study using a $10^7$ organism load of MRSA detected its survival on dry surfaces for several days to almost two weeks, while another similar study concluded that $10^8$ MRSA and other Staphylococcus aureus strains can survive on dry surfaces for months. The reasons for the contradictory information are many. Most of the studies reviewed used different types of surfaces, seeding protocols, organism loads, sampling techniques, growth media, means of assessing growth, and environmental conditions (temperature, humidity, etc.). For the study above in which survival was less than two weeks, organism suspensions were seeded and began drying immediately. In the second study with extended survival, 1 mL of organism suspension was placed in a bottle and the opening was plugged with cotton. Drying was not complete for 10 days, so the extended survival could easily be attributed lack of immediate dessication as well as the higher organism load. Further, within the species Staphylococcus aureus there are many strains, and the selection of a particular strain may influence survival time. Indeed, in one of the studies cited above, the MRSA strain selected by the authors had a substantially longer survival time than the MSSA strain they selected for comparison. When the authors of this study did a follow-up study of five different MRSA strains, they found that MRSA strains associated with outbreaks had better survival times than sporadic MRSA isolates. Therefore, there may be significant variation in survival times between MRSA strains that also have clinical relevance.

There are some common themes in the literature, however. Organism recovery is highly dependent on initial organism load and sampling conditions, with higher initial organism loads showing extended survival. Variables that contributed to increased survival include lower temperatures, humidity >70% and surfaces that offered “pores” into which organisms could embed. One study verified the presence of organisms on seeded surfaces not only with colony counts but also with scanning electron microscopy. Microscopic physical features of inanimate object surfaces that allow organisms to penetrate the surface and perhaps escape dehydration were associated with increased organism visually and by colony count.

Data on survival of MRSA on fomites directly associated with clinical laboratory practice was not found in a comprehensive literature search. In clinical laboratories, there are three MRSA sources of major concern: blood specimens, body fluid specimens, and microbiological cultures. A 0.5 McFarland suspension is a standard laboratory organism load for microbiological manipulation, and it will contain millions of CFUs. Patients with clinically important sepsis have more than 30 CFUs/mL of bacteria in their blood, and acquisition of MRSA into wounds from skin is associated with >200 CFUs. Therefore, a serum suspension containing approximately 2,000 CFUs/ml and a whole blood suspension with approximately 50 CFUs/mL could be used to mimic body fluid and blood splashes, respectively.

Also in the clinical environment there is concern about the transmission of MRSA from “high touch” fomites such as stethoscopes. Sampling these types of objects will help to determine if strains of MRSA can survive on them and if MRSA is capable of being transferred from the hospital into the environment. Clearly if MRSA is being transferred out of the hospital by objects, it poses a threat to society because it can be spread into the community.

**MATERIALS AND METHODS**

Two standard organisms were used from the American Type Colony Collection (ATCC): methicillin-resistant Staphylococcus aureus (MRSA) strain #43300 and methicillin-sensitive Staphylococcus aureus (MSSA) strain #25923. BBL® CHROMagar® for MRSA was used to select for and identify methicillin-resistant Staphylococcus aureus. BBL® trypticase soy (TS) broth and agar were used to dilute and quantify organisms.

Human serum and whole blood anticoagulated in acid/citrate/dextrose were collected by standard phlebotomy from a healthy volunteer. Squares of glass and floor vinyl and a melamine countertop scrap were purchased from a home improvement store.

The nature of the study was explained to Salisbury University respiratory therapy and nursing students, and they were told participation was voluntary and anonymous. Faculty collected student stethoscopes in class on a cart without identifying them in any way, and the stethoscopes were returned to class after swabbing.

Sterile TS broth was used to make a standardized inoculum (0.5 McFarland) from stock broth solution of MRSA. Serial dilutions were made of the 0.5 McFarland standard (1:10, 1:100, 1:1,000, and 1:10,000) using broth. With a 0.001 inoculating loop, each dilution was streaked onto a separate TSA plate and incubated overnight at 37°C. The plate with
the best countable colonies was used to determine the total colony forming units (CFUs)/mL in the 0.5 McFarland standard. The 0.5 McFarland standard was then diluted with whole blood to obtain a suspension with 50 CFUs of MRSA/mL and with serum to obtain a suspension with 2,000 CFUs of MRSA/mL.

Vinyl floor tiles, glass plates, and melamine countertop material were divided into squares and each square was labeled with sequential numbers. To mimic splashes on each of the three surfaces, the following were pipetted and allowed to air dry:

- Into each of 36 squares (5 cm x 5 cm), 0.5 ml of the MRSA 0.5 McFarland standard with 110,000,000 CFUs/mL (55 million CFUs/square)
- Into each of 36 squares (2.5 cm x 2.5 cm), 0.1 mL of serum with 2,000 CFUs/mL MRSA (200 CFUs/square)

50 CFUs/mL MRSA (5 CFUs/square)

To standardize sampling of detectable MRSA remaining in the squares, 100 µl of sterile water were pipetted onto the square to be sampled. A sterile swab was used to absorb the water and swab the surface. Swabs were then inoculated onto CHROMagar® along with MRSA (a positive control) and MSSA (a negative control). Agar plates were incubated at 37ºC and read after 24-48 hours. Positive growth was detected as visible pink colonies. This process was repeated on one square at a time for remaining squares until no MRSA was detectable or until all squares were sampled. Initially, samples were taken daily, but later samples were spaced with several days in between. Examples of seeded fomites and an inoculated plate are shown in Figure 1.

To sample stethoscopes, a sterile swab was dipped into an uninoculated TS broth tube and the stethoscopes were swabbed in the areas which are most likely to frequently come into contact with human skin (near the earpiece, front and back of the diaphragm). CHROMagar® plates were divided into separate quadrants and a different stethoscope sample was swabbed onto each quadrant. A positive and negative control were included on each plate. Agar plates were incubated at 37ºC and read after 24-48 hours. Positive growth was detected as visible pink colonies.

**RESULTS**

As shown in Table 1, live MRSA from the 0.5 McFarland Standard (110 million CFUs/ml) was demonstrated for 60 days, after which all samples had been taken. No live MRSA was recovered from whole blood (50 CFUs/ml), while MRSA in serum (2,000 CFUs/ml) survived at different rates on the different surfaces: 41 days on glass, 45 days on tile, and at least 60 days on countertop.

Table 2 shows that MRSA was present on 3 out of 15 stethoscopes from respiratory therapy students and 2 out of 18 from nursing students for an overall positive rate of 5 out of 33 or 15%.

**DISCUSSION**

Survival of a 10⁷ organism load of dried MRSA for up to about two weeks was shown in a previous study using a curtain, a plastic patient chart, and a laminated table as fomites when organism was seeded and dried immediately. The exact strains used in this study were not given. Our maximum organism load was 5.5 x 10⁷ and we also allowed samples to dry immediately, so based on the survival times...
quoted, our samples were set up for about a month of daily sampling. However, because of the unexpected duration of survival, the later samples were spaced with several days in between to allow the surfaces to be sampled for a total of 60 days.

Survival of MRSA in whole blood on environmental surfaces was not demonstrated. Since these samples had the smallest organism load, this result is not unexpected. Further, the nature of the dried blood made sampling difficult. It is likely, then, that transmission of MRSA from dried blood on objects would be poor.

Serum suspensions with a higher organism load than the whole blood did survive, but at different rates on the different surfaces. Although the countertop serum samples persisted until day 60, the colony count was very low, so additional survival is probably not more than a week or two. Still, this is well beyond the survival expected and indicates that dried body fluids contaminated with MRSA could be a significant source of MRSA transmission. We note, however, the heterogeneity of the term “body fluids”. Sterile serum without immunocompetent cells and with low levels of immune mediators would be expected to overestimate MRSA survival in other types of fluids. However, while the dose of 200 CFUs is considered infectious, it was shown to be the minimum infectious dose on compromised human skin, and many body fluids could have higher organism loads.

The 0.5 McFarland standard samples survived for the entire 60 days on all three surfaces with too many colonies to count remaining. Further studies will be needed to determine the total survival time, and it may significantly exceed 60 days. Since this was the highest organism load and it was in a nutrient medium, extended survival was to be expected. This sample has the fewest implications for the community as the high organism load would only be encountered in laboratories. It does, however, indicate that strict adherence to safety protocols in labs is vital.

Stethoscopes are not objects commonly used in the clinical laboratory, but they are considered “high touch” fomites that might provide surrogate data about the survivability of MRSA on high touch laboratory fomites composed of similar materials. We also had a ready source of stethoscopes that had been removed from the clinical environment. A previous study swabbed 55 stethoscopes from community pediatric clinics and showed that MRSA was present on 7.4%. Since the stethoscopes in this study were from nursing and respiratory therapy students and only 33 were sampled, statistically significant comparisons cannot be made. However, a higher contamination rate may be expected in this research because of significant contact of nursing and respiratory therapy students with hospital inpatients. This information indicates that further study on “high touch” laboratory objects such as microscopes and pipettes might be useful.

### Table 1. Survival time in days of MRSA on fomites

<table>
<thead>
<tr>
<th>Fomite</th>
<th>0.5 McFarland Standard 110 million MRSA CFUs/ml in broth sample seeded for 55 million CFUs/square</th>
<th>Human serum 2,000 MRSA CFUs/ml seeded for 200 CFUs/square</th>
<th>Human whole blood 50 MRSA ml seeded for 5 CFUs/square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melamine counter top</td>
<td>≥60*</td>
<td>≥60*</td>
<td>0</td>
</tr>
<tr>
<td>Vinyl floor tile</td>
<td>≥60*</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Glass</td>
<td>≥60*</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>

*Growth was positive at 60 days at which time all samples were taken. CFU = colony forming unit
This data suggests that different concentrations of MRSA can survive for a much longer time on inanimate objects than was expected prior to conducting this research study. To date, there is no universally accepted method for sampling fomites, and wide variation in organism recovery has been shown depending on sampling technique. The study cited above which showed about two weeks of survival on sampled surfaces used contact agar plates while we applied a standard amount of sterile water and swabbed the surfaces. It is possible that rehydrating the sample not only allowed better swab sampling but also improved growth of dried organism. This may have implications for identifying which fomites are more likely to transmit MRSA. Wet areas such as bathrooms, gyms, and showers may contain surfaces more likely to transmit MRSA. However, our study suggests that MRSA survival on stethoscopes is occurring, so inherently dry surfaces are not necessarily without risk.

The MRSA organism strain we selected was the strain cited in the BBL CHROMagar package insert as the test organism used to verify agar performance. While this strain gave us confidence that use of this medium was justified in the project, at this time we do not know how much influence this particular strain had on the extended survival and if survival would be significantly different with other strains, as implied by other studies mentioned earlier.

This research supports the importance of decontamination to help reduce the spread of highly pathogenic infections, particularly since survival of MRSA was much longer than expected.

Further studies will be needed to determine the exact survival time of MRSA on the various fomites we selected, and survival studies could be done using other strains. In addition, since the physical nature of surfaces contributes to organism survival, there are many more potential fomites to survey. Given that there is no accepted standard for seeding inanimate objects or assessing organism survival, the contradictory data available in the literature is probably to be expected, and the topic will continue to have opportunities for further study.

Clin Lab Sci encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to david.mcglasson@lackland.af.mil. In the subject line, please type “CLIN LAB SCI 22(1) RR DAVIS”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

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Mechanisms of Resistance for *Streptococcus pyogenes* in Northern Utah

RYAN A ROWE, RYAN M STEPHENSON, DESTRY L EAST, SCOTT WRIGHT

**OBJECTIVE:** The purpose of this study was to 1) determine the rates of penicillin and erythromycin resistance among *Streptococcus pyogenes* isolates in northern Utah, and 2) determine the genotype of the erythromycin resistant strains, thereby providing information regarding the mechanism of the resistance.

**DESIGN:** Seven hundred thirty-nine isolates of *S. pyogenes* were identified on 5% Sheep Blood Agar. Susceptibility to erythromycin and penicillin was performed using Muller-Hinton blood agar. All isolates resistant to erythromycin were then genotyped using PCR primers specific to one of the following: *mefA* gene, indicating the mechanism of resistance was an efflux pump; *ermA* gene, in which the mechanism was inducible methylation of the ribosomes; and *ermB* indicating constitutive methylation of the ribosomes.

**LOCATION:** This study was conducted at Weber State University, in the Department of Clinical Laboratory Sciences.

**PATIENT SAMPLES:** Samples were collected from 9 clinics ranging from North Ogden to Taylorsville, Utah. All samples were previously tested positive for *S. pyogenes* by the clinic from where the samples were collected.

**RESULTS:** Of the 739 *S. pyogenes* isolates tested, 2.4% were resistant to erythromycin with no resistance observed to penicillin. Of the strains that displayed some degree of resistance, the gene frequencies observed were as follows: 48.1% *mefA*, 26.0% *ermA*, 3.7% *ermB*, and 22.2% multiple genes.

**CONCLUSION:** The most common genotype was *mefA*, indicating that the efflux pump (M phenotype) is the most common mechanism in the surveyed area, followed by *ermA*, which produces the inducible methylating enzyme. A significant number of isolates was also observed to express both the efflux pump and the constitutive methylating enzyme.

**ABBREVIATIONS:** ATCC = American Type Culture Collection; CLS = clinical laboratory science; CLSI = Clinical Laboratory Standards Institute; IU = international unit; MLSB = macrolide, lincosamide, streptogramin B; PCR = polymerase chain reaction; SBA= sheep blood agar; WSU = Weber State University.

**INDEX TERMS:** erythromycin; erythromycin resistance mechanisms; PCR; penicillin; *S. pyogenes*.


*Ryan A Rowe MLT(ASCP), Ryan M Stephenson MLT (ASCP), Destry L East MLT (ASCP), and associate professor Scott Wright MS CLS(M)(NCA) are of the Clinical Laboratory Science Program, Department of Health Professions, Weber State University, Ogden UT.*

**Address for correspondence:** Scott Wright MS CLS(M)(NCA), associate professor, Clinical Laboratory Science Program, Department of Health Professions, Weber State University, 3905 University Circle, Ogden UT, 84408-3905. (801) 626-6716. swright@weber.edu.

**ACKNOWLEDGEMENTS:** We would like to express our gratitude to the Clinical Laboratory Sciences Department faculty and staff for their endless help and support. We would also like to thank the clinics involved in the collection of samples for testing. We would like to thank our colleagues Todd Keskey, Bruce Jolly, Lauren Jackson, and Zach Robinet for helping us collect and isolate resistant strains. This project was funded through the Office of Undergraduate Research at WSU with a grant from the Denkers Undergraduate Research Scholarship.

This information was presented as an oral presentation at the Weber State University Undergraduate Research Symposium and
Streptococcus pyogenes is the most common cause of acute pharyngitis and can also be the cause of many other diseases, including bacteremia and necrotizing fasciitis. Because most strains are sensitive to penicillin, S. pyogenes infections are preferably treated with a drug from the penicillin family. If the patient is allergic to the drug, antimicrobials called macrolides, such as erythromycin, are used. Macrolide-resistant strains of S. pyogenes have become a significant concern for healthcare providers. In Europe, studies have reported resistance to macrolides in more than 10% of strains tested from patients with S. pyogenes infections. Studies conducted in Spain and Italy have reported resistance rates of 25% and 38%, respectively. Over-prescription of macrolides is one suspected cause of this resistance. Resistance in the United States has been reported as being somewhat lower than in European countries, ranging from three percent to nine percent. Despite this relatively low rate, it continues to be a concern in the US due to the rapid increase of the resistance seen in Europe over just a few decades.

S. pyogenes resists the activity of macrolides via two predominant mechanisms. Some resistant strains utilize an efflux mechanism, where the bacterium utilizes a membrane-associated protein that pumps the macrolide out of the cell, severely reducing the antimicrobial effect. This membrane protein is specific for macrolides. When the efflux mechanism is present in a macrolide-resistant strain it is referred to as the M phenotype. S. pyogenes strains expressing the efflux pump are resistant to erythromycin, but they are susceptible to clindamycin, a drug from the lincosamide family and streptogramin B. Macrolides, lincosamides, and streptogrammin B are destructive to bacteria by binding to ribosomes and interfering with protein translation. The efflux pump (M phenotype) is associated with the presence of the mefA gene.

The second mechanism of resistance is the modification of the bacterial ribosomes. Bacteria with this mechanism produce an enzyme that adds a methyl (-CH₃) group on the ribosome. This methylation slightly changes the shape of the ribosome and reduces the affinity of the drug for the ribosome. The characteristics displayed by this mechanism are referred to by the MLS₉ phenotype.

There are two types of MLS₉, inducible and constitutive. These MLS₉ phenotypes can be easily distinguished by utilizing the D-test, or double disk diffusion test with a macrolide and a lincosamide. The inducible MLS₉ requires the presence of a specific antimicrobial to induce the production of the enzyme. In the case of S. pyogenes, the macrolide erythromycin can elicit the production of the enzyme whereas the lincosamide clindamycin cannot. The bacterium is resistant to the lincosamide only in the presence of the enzyme inducing macrolide. Inducible MLS₉ correlates with the ermA gene. The constitutive MLS₉ mechanism continually produces the enzyme, and can therefore continually resist any ribosome targeting antimicrobial. Constitutive MLS₉ expresses resistance to erythromycin and clindamycin, and is associated with the ermB gene.

Typically, all of these mechanisms for macrolide resistance are represented in a given patient population. However, which mechanism is predominant varies from place to place. The efflux pump (mefA gene) is the most common in Spain. A French pediatric study found 69.4% of the erythromycin resistance to be caused by the constitutive MLS₉ (ermB gene). In the United States, the efflux pump (mefA) has been previously reported to account for 43%, while inducible MLS₉ (ermA) accounts for 46%, with constitutive MLS₉ (ermB) being much less at 8.5%. The purpose of this study is to determine the rates of each mechanism of erythromycin resistance in northern Utah.

**MATERIALS AND METHODS**

**Collection and isolation.** Throat swabs were collected between October 2007 and March 2008 from 9 different clinics (Figure 1) covering a geographical area between North Ogden, and Taylorsville, Utah, which is about 50 miles south. The collection and isolation of S. pyogenes was performed in conjunction with another research group within the CLS department at WSU. All of the isolates were recovered from pharyngeal swabs collected from symptomatic patients. Clinic laboratories tested for S. pyogenes using point of care Strep tests. The swabs were then streaked onto five percent Sheep Blood Agar (SBA) plates (Hardy Diagnostics). Colonies that were translucent, β-hemolytic, and catalase negative were then subcultured to another SBA agar plate. Identification of the species was done using three criteria: β-hemolysis, susceptibility to bacitracin disk (0.04 IU), and a positive streptolysin-O stab.

**Susceptibility testing.** Susceptibility testing was performed using a Kirby Bauer disk diffusion method on Muller-Hinton agar plates, supplemented with five percent sheep blood (Hardy Diagnostics) according to CLSI M2-A9 guidelines.
Each isolate was put into Columbia broth to a concentration of 0.5-0.9 McFarland units, and then swabbed onto Muller-Hinton blood agar bi-plates. A 10 IU penicillin and a 15 µg erythromycin disk were placed onto the swabbed area and then placed in an air incubator at 37°C for 16-24 hours.

After incubation, the zones of inhibition to each disk were measured using a micrometer. The size of the zone was used to determine if the strain was resistant (≤ 15 mm), intermediate (16-20 mm), or susceptible (≥ 21 mm) using the zones of inhibition listed by the antimicrobial manufacturer.

**Controls.** Several control strains were used in this study. For the bacitracin disks, *S. pyogenes* ATCC 19615 was used for the susceptible control, and *Streptococcus agalactiae* ATCC 12386 for the resistant control (MicroBiologics.com). For the erythromycin and penicillin susceptible control, *S. pyogenes* ATCC 19615 was used. For the erythromycin resistant controls representing each mechanism, strains of *S. pyogenes* were provided courtesy of the Doern research laboratory at the University of Iowa. There was no resistant control for penicillin.

**Phenotyping.** For the D-test, erythromycin resistant samples were once again put into a Columbia broth to a concentration of 0.5-0.9 McFarland units. The samples were swabbed onto a Muller-Hinton blood agar plate. The macrolide used for this test was a 15 µg erythromycin disk and the lincosamide used was a 2 µg clindamycin disk. The disks were placed 16-21 mm apart from each other, measuring from the center of each antimicrobial disk. Plates for the D-test were placed in the air incubator at 37°C for 16-24 hours. Again, the size of the zone of inhibition was used to determine if the strain was resistant, intermediate, or susceptible using the zones of inhibition included with the antimicrobials. If the isolate was resistant to erythromycin (≤ 15 mm) and susceptible to clindamycin (≥ 19 mm), we recorded it as the M phenotype (efflux pump). The presence of a D shape around the susceptible region of clindamycin was the inducible MLS\textsubscript{B} phenotype. Resistance to erythromycin (≤ 15 mm) and clindamycin (≤ 15 mm) was recorded as the constitutive MLS\textsubscript{B} phenotype.

**DNA extraction.** DNA was extracted from strains showing a zone that was measured to be either intermediate or resistant to erythromycin. The extraction was performed using Qia-gen QIAamp\textsuperscript{®} DNA mini kit, following the manufacturer’s protocol for DNA extraction from bacterial colonies.\textsuperscript{9}

**PCR amplification and detection.** PCR amplification was used to detect *mefA*, *ermA*, and *ermB* genes. Previously published primers (*mefA*, 5’-AGT ATC ATT AAT CAC TAG TGC-3’ and 5’-TTC TTC TGG TAC TAA AAG TGG-3’; *ermA*, 5’-GCA TGA CAT AAA CCT TCA-3’ and 5’-AGG TTA TAA TGA AAC AGA-3’; and *ermB*, 5’-CGA GTG AAA AAG TAC TCA ACC-3’ and 5’-GCG GTG TTT CAT TGC TTG ATG-3’)\textsuperscript{5} and thermal cycler conditions\textsuperscript{1} were used. Each extracted sample was tested for each of the three genes separately. The PCR reaction concentrations consisted

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**Figure 1.** Number of erythromycin resistant and intermediate strains listed by clinic location
of the following: 20 pM primers, 200 µM of each deoxynucleotide, 1.5 mM MgCl₂, 160 mM (NH₄)₂SO₄, 670 mM Tris-HCl (pH 8.8), 0.1% Tween-20, 2.5 U DNA polymerase (Bioline), and 1 µL of extracted DNA. Amplification products were separated by electrophoresis on a 1% agarose gel (150V for 30 min), and stained with ethidium bromide. The presence of the gene was determined by the presence of bands with the expected molecular sizes of 348, 206, and 616 base pairs for \( \text{mefA} \), \( \text{ermA} \), and \( \text{ermB} \) genes, respectively. 

Control strains were used to confirm the accuracy of the PCR amplification: \textit{S. pyogenes} 8084 for \( \text{mefA} \), \textit{S. pyogenes} 609 for \( \text{ermA} \), and \textit{S. pyogenes} 6945 for \( \text{ermB} \) (provided courtesy of the Doern research laboratory at the University of Iowa).

**RESULTS**

A total of 739 \textit{S. pyogenes} isolates were identified and tested. Of these there were 36 that showed some degree of resistance. There were 18 resistant isolates, and 18 intermediate isolates (Figure 1). This equals a resistance rate of 2.4% and an intermediate rate of 2.4% (Table 1). All isolates showed susceptibility to penicillin.

**Genotypic observations.** After amplification, 27 isolates out of 36 showed the presence of at least one of the three probed genes. Of those 27, 13 displayed only the \( \text{mefA} \) gene (48.1%), 7 displayed only the \( \text{ermA} \) gene (26.0%), 1 displayed only the \( \text{ermB} \) gene (3.7%), 4 displayed both the \( \text{mefA} \) and \( \text{ermB} \) genes (14.8%), 1 displayed both the \( \text{mefA} \) and \( \text{ermA} \) genes (3.7%), and 1 displayed all three genes (3.7%). One resistant sample that showed constitutive \( \text{MLS}_B \) phenotypic characteristics had no gene amplified (see Figure 2 and Figure 3). There were eight intermediate isolates that did not show the presence of any gene after PCR amplification.

**Phenotypic observations.** Phenotypes were measured and recorded on 11 of the 36 erythromycin resistant and intermediate isolates. There were four samples (36%) that expressed the M phenotype (erythromycin resistant and clindamycin susceptible). There were four samples (36%) that displayed the inducible \( \text{MLS}_B \) phenotype (erythromycin resistant and inducible clindamycin resistance). The remaining three samples (27%) displayed the constitutive \( \text{MLS}_B \) phenotype (resistant to both erythromycin and clindamycin). The positive correlation between the phenotypes and genotypes of these samples are as follows: M phenotype, three of four contained the \( \text{mefA} \) gene (75%); inducible \( \text{MLS}_B \) phenotype, three of four contained the \( \text{ermA} \) gene (75%); constitutive \( \text{MLS}_B \) phenotype, one of three contained the \( \text{ermB} \) gene (33%). Of the 11 isolates with recorded phenotypes, 2 erythromycin

<table>
<thead>
<tr>
<th>Tested Samples</th>
<th>Isolates</th>
<th>Resistant</th>
<th>Resistance (%)</th>
<th>Intermediate</th>
<th>Intermediate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Ogden</td>
<td>98</td>
<td>5</td>
<td>5.1%</td>
<td>5</td>
<td>5.1%</td>
</tr>
<tr>
<td>Ogden</td>
<td>134</td>
<td>1</td>
<td>0.7%</td>
<td>1</td>
<td>0.7%</td>
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<tr>
<td>Pediatric</td>
<td>36</td>
<td>0</td>
<td>0.0%</td>
<td>1</td>
<td>2.8%</td>
</tr>
<tr>
<td>South Ogden</td>
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<td>2.4%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
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<td>33.3%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Davis</td>
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<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Tanner</td>
<td>142</td>
<td>2</td>
<td>1.4%</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>Redwood</td>
<td>108</td>
<td>1</td>
<td>0.9%</td>
<td>3</td>
<td>2.8%</td>
</tr>
<tr>
<td>Taylorsville</td>
<td>166</td>
<td>6</td>
<td>3.6%</td>
<td>7</td>
<td>4.2%</td>
</tr>
<tr>
<td>Total</td>
<td>739</td>
<td>18</td>
<td>2.4%</td>
<td>18</td>
<td>2.4%</td>
</tr>
</tbody>
</table>
intermediate isolates failed to amplify any gene. Their phenotypes were documented as M and inducible MLSB, respectively. There were two samples that amplified a gene other than the one predicted by the phenotype.

DISCUSSION
A significant finding from this study was that all S. pyogenes isolates were susceptible to penicillin, verifying its continued effectiveness against the organism. Previous studies have also reported little to no penicillin resistance in S. pyogenes. The results show that the efflux pump (mefA gene) was the most prevalent mechanism (48.1%) of the erythromycin resistant strains. This is higher than the percent found in a previous published study done nationwide. However, the percent of the inducible MLSB (ermA) was found to be lower in northern Utah than nationwide.

Some samples also showed multiple genes. Pairing of ermA/mefA and ermB/mefA in this study was observed at a higher percent than the national average (Figure 4). This survey also saw a lower percent of ermA/ermB pairings than the national average, as well as a decrease in the rate of isolates showing all three genes compared to the national average.

The national study showed only 7 samples out of 256 collected from the mountain area to be resistant to macrolides, displaying a 2.7% resistance rate. The same national survey showed that from 129 resistant strains collected nationwide, 11 showed the presence of ermB for an 8.5% occurrence whether paired with another gene or not. In this current study, an increased percentage of resistant strains was observed presenting the ermB gene (31.6%), contrasting the rate (8.5%) found in the national study (Figure 4). This may be noteworthy due to the fact that ermB correlates with the constitutive MLSB phenotype, which is the only known erythromycin resistant mechanism that expresses resistance to lincosamides and streptogramin B without the inducing macrolide present. An increased frequency of this gene could limit the antimicrobials that healthcare providers use in the case of invasive streptococcal infection. The overall erythromycin resistance rate of 2.4% is low enough to suggest that erythromycin and other macrolides are still viable options in the treatment of S. pyogenes for patients with penicillin allergies. Efflux (mefA) and inducible MLSB (ermA) are the primary mechanisms causing the resistance in northern Utah. Constitutive MLSB (ermB) was present in 18.5% of resistant isolates, making it a significant contributor to the resistant population as well.
Because each mechanism is well represented in the resistant population, potential for expansion exists for each type. Continued utilization of penicillin as the first choice in the treatment of *S. pyogenes* will likely help to limit the potential for increased macrolide resistance in northern Utah.

There were eight intermediate isolates that did not show the presence of any gene after PCR amplification, and one strain that showed constitutive MLS$_B$ phenotype, also with no gene product detected. One possible explanation for this may be due to another, less frequently encountered mechanism that was not included in this study.

**CONCLUSION**

The rates of resistance for *S. pyogenes* to erythromycin in the northern Utah area are still at a relatively low level. Penicillin based antimicrobials should still be the first drug of choice when prescribing antimicrobials for treatment against this organism. The most common mechanism responsible for the resistance in the population of patients surveyed in this study was the efflux pump due to the presence of *mefA* gene (M phenotype). Coupling of *mefA* and *ermB* genes (efflux with constitutive ribosomal modification) suggests a greater presence of constitutive MLS$_B$ phenotype will be seen in clinical settings when testing for mechanisms using the D-test. Knowing the mechanisms of resistance is useful in determining how to counteract the resistance and will influence the development and future administration of antimicrobials.

*Clin Lab Sci* encourages readers to respond with thoughts, questions, or comments regarding this article. Email responses to david.mcglasson@lackland.af.mil. In the subject line, please type “CLIN LAB SCI 22(1) RR WRIGHT”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

**REFERENCES**

FOCUS: BODY FLUIDS

Introduction

LEILANI COLLINS

ABBREVIATIONS: AIDS = acute immunodeficiency syndrome; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; RBC = red blood cell.

INDEX TERMS: body fluids; cerebrospinal; serous; synovial.


LEARNING OBJECTIVES
1. Discuss the necessity of performing the cell counts and slide preparation on body fluids as soon as possible after collection.
2. Explain the value of a monolayer slide preparation in determining cell morphology in body fluids.
3. Explain why the presence of serous and synovial fluids in quantities sufficient to sample is an indication of a disease process.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, Memphis TN.

Address for correspondence: Leilani Collins MS MT(ASCP)SH CLS(NCA), associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, 930 Madison Avenue, Suite 670, Memphis TN 38163. (901) 448-6299. lcollins@utmem.edu.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is the Focus: Body Fluids guest editor.

This series of articles will address the performance of cell counts and differential cell counts on the three most common categories of body fluids: cerebrospinal fluid (CSF), serous or body cavity fluids (pleural, pericardial, peritoneal), and synovial or joint fluids. Each has unique characteristics and cell counts, and differentials are performed for different purposes on each one. When a fluid is received in the laboratory, information that can be helpful in determining the cell counts is obtained from the gross appearance of the fluid. A point to be remembered is that the specimen is obtained by a physician who also observes the gross appearance of the fluid. This is especially significant in CSF if only one tube is obtained and it is bloody. Every body fluid should be examined—cell count performed and slide for evaluation of cell morphology prepared—immediately after collection since cells, especially neutrophils, begin disintegrating within 30 minutes.

Cytocentrifuge concentration of cell preparations improves cell identification over attempting to differentiate cells while performing hemacytometer cell counts. With appropriate dilution, a monolayer slide can be prepared to enhance morphology of cells. Cytocentrifuge concentration provides enough nucleated cells to perform a 100-cell differential count if the nucleated cell count is greater than 3/μL. Since there will be some cell yield even if the cell count is zero, important diagnostic information can be obtained especially in leukemic patients when blast cells may be present. The presence of red blood cells (RBCs) in fluids can interfere with nucleated cell morphology due to crowding and distortion of significant cells. Cytocentrifuge preparations also help identify eosinophils in urine and lupus erythematosus cells in serous and synovial fluids.

Many conditions are diagnosed based on findings in a body fluid. Since CSF is a normal fluid for which reference ranges are defined, any increase in the nucleated cell count may herald meningitis, whether viral or bacterial. Blast cells or lymphoma cells in CSF of a patient previously diagnosed with acute leukemia or lymphoma may be the first indication of relapse. In patients with HIV or AIDS and neurologic symptoms, the finding of cryptococcal organisms may explain the patient’s symptoms.

The presence of serous and synovial fluids in quantities sufficient to sample is an indication of a disease process involving a body organ or a joint. It is helpful to identify effusions in body cavities as transudates or exudates to aid in identification of the pathologic condition responsible for the excess fluid. This delineation can be confirmed by the types of cells seen in the fluid. Often, cancer metastasizes to the lungs and liver so metastatic tumor cells as well as primary tumor cells may be seen in exudative effusions.

Synovial fluids will contain neutrophils in the presence of inflammation or infection. If inflammation in the joint is due to gout or pseudogout, the characteristic crystals for these conditions will be present.

It is important for laboratory personnel performing body fluids to be familiar with methods for performing accurate nucleated cell counts, preparing cytocentrifuge cell concentrations, and identifying significant cell morphotypes in fluids.
Examination of Body Fluids: Evaluating Gross Appearance; Performing Cell Counts

LEILANI COLLINS

ABBREVIATIONS: CNS = central nervous system; CSF = cerebrospinal fluid; RBC = red blood cell; WBC = white blood cell.

INDEX TERMS: body fluids; cerebrospinal; serous; synovial.


LEARNING OBJECTIVES

1. Describe the normal appearance of cerebrospinal fluid, serous fluids, and synovial fluid.
2. Define gross findings that distinguish a traumatic tap from a pathologic bleed when a bloody CSF is encountered.
3. Name laboratory findings that aid in identifying a serous fluid as a transudate or exudate.
4. Describe how the appearance of a fluid can be used to determine the correct dilution for accurate cell counts.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, Memphis TN.

Address for correspondence: Leilani Collins MS MT(ASCP)SH CLS(NCA), associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, 930 Madison Avenue, Suite 670, Memphis TN 38163. (901) 448-6299. lcollins@utmem.edu.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is the Focus: Body Fluids guest editor.

Evaluating gross appearance: CSF

Spinal fluid is the only fluid that is normally available in quantities sufficient to sample. Adults have 90-150 mL of cerebrospinal fluid (CSF) and neonates 60-90 mL. The spinal fluid bathes the brain and spinal column providing nutrients to, removing waste products from, and acting as a protective cushion for the central nervous system (CNS). Since this fluid circulates around the brain and spinal column, it is an excellent source of information for the condition of the CNS. CSF is collected by lumbar puncture and, ideally, is placed into three or four sterile tubes labeled 1, 2, 3, and 4 in the order in which it is collected. If multiple tubes are received and multiple tests are ordered, tube #1 should be used for chemistry and immunology testing, tube #2 for microbiology, tube #3 for cell counts and differential, and tube #4 for any other tests.

The normal appearance of CSF is colorless and crystal clear, resembling water. A cloudy or turbid fluid indicates increased WBCs or protein. A bloody fluid can indicate a traumatic tap in which a blood vessel is inadvertently punctured in the process of collection or blood can mean an intracranial hemorrhage or pathologic bleed. If multiple tubes are collected, there is clearing of the blood from the first tube to the last tube collected in the case of a traumatic tap but a consistent amount of blood in all tubes in the case of a pathologic bleed. If only one tube containing bloody fluid is obtained, an aliquot of the fluid should be centrifuged and the appearance of the supernatant examined. The supernatant will be colorless and crystal clear in the case of traumatic tap and xanthochromic (yellow) to hemolyzed (red) if a pathologic bleed has taken place.

Serous fluid

The heart, lungs, and intestine—organs that move—are each enclosed in a sac. Between the sac and the organ there is a single layer of cells called mesothelial cells. Serous fluid flows through the “space” between the organ and its sac, allowing the organ to move smoothly against the sac. Serous fluid does not normally exist in quantities sufficient to sample so when it arrives in the laboratory to be analyzed—usually in very large quantities—it indicates an abnormal condition and is termed an effusion. An effusion is determined to be
FOCUS: BODY FLUIDS

a transudate or an exudate using several visual and chemical findings (Table 1). Transudates indicate accumulation of fluid due to a benign systemic process such as chronic heart failure or cirrhosis. Transudates are created when fluid is filtered through vessel walls. Transudates contain few chemical and cellular elements and have a low specific gravity.

Exudates indicate a localized condition that affects the surfaces of organs directly such as malignancies, infections, or trauma. Because these conditions cause fluid release due to vessel damage, exudates contain a high concentration of protein and cellular elements and have a high specific gravity.

Normal serous fluid is pale yellow and clear. Transudates are pale yellow and hazy to cloudy depending on the number of cells present. Exudates are cloudy and may be bloody. In pleural fluids, chylous effusions are milky white or yellow-bloody and indicate damage or obstruction to the thoracic duct secondary to trauma, malignancy, or congenital abnormality; pseudochylous effusions are also milky or green with a metallic sheen, contain cholesterol crystals and indicate chronic inflammation.

Synovial fluid
Synovial (joint) fluid is normally not present in amounts sufficient to sample, but volume increases in inflammation. When it does not contain significant numbers of cells or crystals, synovial fluid is pale yellow and clear. Because this fluid contains hyaluronic acid, it is viscous and is difficult to work with unless it is liquefied with hyaluronidase, an enzyme that breaks down the hyaluronic acid. Synovial fluid that contains large numbers of crystals may appear “milky”.

Performing cell counts
All samples should be thoroughly mixed prior to performing cell counts or preparing slides. If the specimen contains clots, tissue particles, or other bodies, that finding should be noted on the report. If those “objects” interfere with the performance of counts, they can be removed prior to performing cell counts or preparing slides.

Cell counts on body fluids are usually performed on the hemacytometer. When performed on cell counting instruments, linearity must be confirmed since very low cell counts can be significant in CSF. Hyaluronidase should be added to synovial fluids before cell counts are performed or cytocentrifuge slides are prepared. When using the hemacytometer to perform cell counts, the dilution and number of squares counted should be adjusted for the number of nucleated cells present, which may be estimated from the specimen’s clarity.

Cells/µL. Cells counted X 10 (depth factor) X dilution factor

While the presence of RBCs in fluids is significant and should be reported in the gross appearance description, the RBC count has no clinical significance. The clinically significant cells are the nucleated cells. When performing cell counts, fluids with low nucleated cell counts may be counted without dilution. If RBCs are present, it is helpful to use a diluting fluid such as acetic acid or Türk’s solution that will lyse the RBCs and enhance the nucleus of nucleated cells. Türk’s solution preparation: Dilute 3 mL glacial acetic acid to 100 mL total volume with deionized water and add two to three drops methylene blue or Giemsa stain. Using calibrated pipettes, dilutions of 1:2, 1:3, and so on can be made to provide accurate cell counts on fluids containing low numbers of nucleated cells. Acetic acid-based diluting fluids should not be used when counting cells in synovial fluids since acetic acid causes the formation of mucin clots.

The dilution needed to perform cell counts can be estimated by the appearance of the fluid using the following criteria:
• Clear: Nucleated cell count will be less than 200/µL; RBC count will be less than 2000/µL. No dilution is necessary; the number of squares counted depends upon

| Table 1. Transudates vs. exudates in serous fluids² |
|---------------------------------|-----------------|-----------------|
| Characteristic                  | Transudates     | Exudates        |
| Color                           | clear/straw     | cloudy/yellow,|
|                                 |                 | amber, grossly |
|                                 |                 | bloody         |
| Specific gravity                | <1.016          | >1.016          |
| Protein                         | <3 g/dL         | >3 g/dL         |
| LDH                             | <200 IU         | >200 IU         |
| Nucleated cell count            | <1000/µL        | >1000/µL        |

Area counted in mm²
the number of cells seen.

- Hazy or cloudy: Nucleated cell count will be greater than 200/µL or RBC count will be greater than 2000/µL. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count.

- Bloody: RBC count will be greater than 5000/µL and there will be distortion of nucleated cells on cytocentrifuge preparations. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count.

- Grossly bloody: RBC count will be ≥1,000,000/µL. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count. To prepare a slide for cellular examination, make a “push” slide and perform a differential on cells pushed out to the end of the smear or on the edges of the smear, not in the body of the smear as on a peripheral blood smear. The larger and more significant cells (including tumor cells) will be pushed out to the end or edges of the smear.

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WORKS CITED
Examination of Body Fluids: Preparation of Slides and Cell Morphology

LEILANI COLLINS

ABBREVIATIONS: ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; CNS = central nervous system; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; RBC = red blood cell; WBC = white blood cell.

INDEX TERMS: body fluids; cerebrospinal; serous; synovial.

LEARNING OBJECTIVES
1. Describe and calculate the proper dilution for preparation of a monolayer cytocentrifuge slide.
2. List cellular findings that may be present in any fluid.
3. Describe lining cells that may be found in CSF, serous, and synovial fluids.
4. Distinguish benign and malignant cells.
5. Compare distinguishing characteristics of significant crystals in synovial fluids.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, Memphis TN.

Address for correspondence: Leilani Collins MS MT(ASCP)SH CLS(NCA), associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, 930 Madison Avenue, Suite 670, Memphis TN 38163. (901) 448-6299. lcollins@utmem.edu.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is the Focus: Body Fluids guest editor.

Specimens concentrated by centrifugation or cytocentrifugation are used to perform differential counts and assess cell morphology on body fluids. Using standard centrifugation, the specimen is centrifuged, the supernatant removed, and a slide made from the buffy coat if RBCs or the concentrated cells of the centrifugate are present. In cytocentrifugation the specimen is transferred to a cytofunnel assembly. The specimen is centrifuged at 1000 RPM for 10 minutes allowing fluid to be absorbed into filter paper and cells to be concentrated in a small area of the slide. The slides prepared by either concentration method should be allowed to air dry and are then stained with Wright or Wright-Giemsa stain prior to examination. If it is not possible to prepare cell concentrations to assess morphology of nucleated cells and cells are differentiated while the cell count is performed, a lysing agent that enhances the nucleus of cells must be used to determine the category of the cells. In this article, preparation of slides refers to cytocentrifugation.

To prepare a monolayer cytocentrifuge slide, which is optimal for nucleated cell identification, use the nucleated cell count (not the RBC count) to determine the saline dilution for the cytocentrifuge preparation. A consistent amount of undiluted or diluted fluid should be used to prepare a cytocentrifuge slide—usually 0.25 mL or five drops. A good monolayer preparation can be obtained if the nucleated cell count is less than 200/µL. If the nucleated cell count is greater than 200/µL, divide the cell count by 100 to obtain the dilution factor.

- Example: Nucleated cell count = 1200/µL.
  - Dilution for cytocentrifuge = 1200/100 = 12 = 1 drop of fluid + 11 drops of saline. Transfer five drops of the diluted fluid to the cytofunnel.

RBCs in quantities greater than 2000/µL distort a concentrated preparation due to crowding. Dilutions should not be based on the RBC count, however, and any lysing agents used to lyse red cells will also distort the morphology of nucleated cells.

Cell morphology in body fluids
The morphology of nucleated cells is the most important
diagnostic factor in any body fluid analysis. While some cells may be found in all fluids, their significance may be different depending on the fluid being analyzed. Normal cells in all fluids are lymphocytes, macrophages, and lining cells. The appearance of these cells can vary depending on the location. For example, macrophages in CSF appear as monocytes, but in serous and synovial fluids they appear as histiocytes. Once a macrophage has ingested cellular material, it is named for the material ingested. For example, a macrophage that has ingested RBCs is termed an erythrophage; if the RBCs have been ingested for more than 72 hours, the iron in the RBCs is visible and the cell is termed a siderophage. The finding of erythrophages and siderophages has different significance in various fluids. They always indicate the attempt of the body to rid a space of RBCs but in CSF they are indications of a pathologic bleed instead of a traumatic tap because the blood would have to be in the CNS for at least 24 hours. Erythrophages in serous or synovial fluids also indicate bleeding in the space around the organ or joint, but the finding of a significant amount of fluid in these spaces is more indicative of a pathologic condition.

Cells found in all fluids

Lymphocytes can be found in any fluid. Usually they appear as normal lymphocytes in peripheral blood. When seen in CSF in viral meningitis, they will demonstrate pleomorphism with variation in size, appearance and basophilia. Macrophages are a normal finding in any fluid. In CSF, they appear as monocytes in peripheral blood unless they have ingested RBCs. In serous and synovial fluids, they are larger with abundant foamy cytoplasm and are known as histiocytes or macrophages.

Neutrophils are not normally present but are present in infection and inflammation. Neutrophils in body fluids are hypersegmented and have prominent filaments.

Eosinophils can be seen in any fluid and usually indicate an allergic response to a foreign substance. Often, basophils are present with eosinophils. Eosinophils are a common finding in CSF from patients with hydrocephalus who have a shunt to drain fluid around the brain to the abdomen.

Bacteria and yeast can be seen in any fluid. When large numbers of neutrophils are seen, a thorough search for bacteria should be made. The finding of intracellular bacteria is significant since that eliminates the possibility of contamination of slides from extraneous sources such as coughs, sneezes, or saliva. All bacteria stain blue or purple with Wright stain and should not be mistaken as Gram positive organisms.

Tumor cells may be seen in any fluid but are rare in synovial fluids. If the presence of tumor cells is suspected, a differential should not be performed on the fluid. Instead, the fluid should be prepared for cytology review and the report should indicate that a cytology report will follow. Tumor cells have some common characteristics though not all tumor cells have the same characteristics. Tumor cells

**Figure 1.** Large macrophage containing erythrocytes, siderotic granules, and one bilirubin (hematoidin) crystal

**Figure 2.** Cryptococcal meningitis
may be large and dark-staining or unevenly stained. Often they are in clumps that are three dimensional or “stacked”. Mitotic figures may be present within the clumps of cells. The cells may have irregular or bizarre nuclear shapes and often the borders of the nucleus may be indistinct, irregular, and disintegrated making it difficult to determine where the nucleus ends and the cytoplasm begins. Nucleoli may be large and prominent. There can be cytoplasmic and nuclear vacuoles. Tumor cells can demonstrate nuclear and cellular molding where the cells line up together, and even demonstrate cannibalism. Tumor cells can take on many unusual shapes and characteristics (pleomorphic) so if cells are encountered that don’t “fit” into a familiar morphotype, suspect tumor cells and defer to cytology review.

Cerebrospinal fluid
The normal nucleated cell count in CSF is <5/µL. If there are fewer than 100 cells on a cytocentrifuge preparation, the cells present should be differentiated and reported as a whole number, not a percentage. Otherwise, 100 cells should be differentiated and reported as a percentage. The predominant cell type in adults is the lymphocyte with few monocytes. In neonates, the predominant cell is the monocyte.

In the case of a traumatic tap, there will be peripheral blood contamination including RBCs and white blood cells (WBCs). There may also be choroid plexus cells, the lining cells of the CNS. These cells are large with abundant lavender cytoplasm and a well-defined purple nucleus on Wright stained slides. They can occur in clumps but the well defined nucleus identifies them as benign cells. If the cartilage of the vertebrae is punctured during the tap, cartilage cells may be seen. These cells are larger than WBCs and have wine-red cytoplasm with a deeper wine-red nucleus. The vertebrae are sites of bone marrow production so it is possible to have bone marrow contamination as part of a traumatic tap. The best indication of bone marrow contamination is the presence of RBC precursors or nucleated RBCs. Any cell that is found in the bone marrow, including megakaryocytes and blasts, can be seen. A differential should not be reported on CSF contaminated with bone marrow but a note that “bone marrow contamination suspected” should be reported instead.

The cellular findings in a pathologic brain bleed include erythrophages and siderophages. See Figure 1. Some siderophages may contain hematoidin or bilirubin crystals, which are golden-yellow rectangular crystals. If a patient has had severe head trauma or brain surgery, neural tissue may be seen. This tissue will appear as clumps of foamy lavender material containing several small, well-spaced nuclei or dendritic cells containing a nucleus with elongated and branched cytoplasm.

Meningitis is the term used for any condition that causes inflammation to the meninges or covering of the brain. In viral meningitis, there will be an elevated nucleated cell count usually in the hundreds/µL. In bacterial meningitis, the nucleated cell count will be extremely elevated, usually in the thousands/µL. In viral meningitis, the predominant cell is the lymphocyte with marked pleomorphism from normal-appearing to reactive and plasma-like lymphocytes. In bacterial meningitis, the predominant cell is the neutrophil and a careful and thorough search should be made for intracellular bacteria since only a small number of bacteria may be present on the initial spinal tap.

Cryptococcal meningitis can be seen in immunocompromised patients. *Cryptococcus neoformans* is an encapsulated yeast. On Wright stain, it may appear as individual organisms appearing separated from each other by unstained capsules or the capsules can take up the stain producing a reddish-purple “starburst” pattern. See Figure 2.

Leukemic meningitis occurs in patients with acute leukemia when leukemic cells are present in the meninges. The blood-brain barrier prevents chemotherapeutic drugs from entering the central nervous system so these patients must receive intrathecal therapy: chemotherapy injected directly into the
spinal column. This is especially common in acute lymphoblastic leukemia (ALL) but may be seen in acute myelogenous leukemia (AML) and lymphoma. If evidence of a traumatic tap is present in a specimen from a leukemic patient, great caution should be taken in reporting blasts in the spinal fluid since blasts seen can be the result of peripheral blood contamination if there are blasts in the patient’s peripheral blood. The spinal tap should be repeated in approximately three days to ascertain the presence of blasts in the CNS.

Carcinomatous meningitis occurs when tumor cells are seen in CSF. These may be the result of primary brain tumors or metastatic carcinoma. The tumor cells seen will have characteristics mentioned earlier in this article.

**Serous fluid**

The presence of serous fluid is indicative of a pathologic condition. Normal cells in serous fluids include lymphocytes, macrophages, and mesothelial cells. Neutrophils are commonly seen in these fluids since an inflammatory or infectious condition is the likely cause of the effusion.

Mesothelial cells are the lining cells found in the pleural, pericardial, and peritoneal cavities. The mesothelium is composed of a single layer of cells and these cells can be seen in clumps or clusters that are only one cell thick. They are large cells and have a “fried egg” appearance with one or more distinct, well-defined oval nucleus or nuclei. They may become very large with many nuclei and abundant cytoplasm. The cytoplasm may contain vacuoles. See Figure 3.

Malignant cells due to either primary or metastatic carcinomas can be found in serous fluids, especially pleural fluids. These cells will have characteristics described earlier. If tumor cells are seen, a differential count should not be performed but the specimen should be prepared for cytological evaluation. See Figure 4.

In the case of a pseudochylous effusion of pleural fluid, cholesterol crystals may be seen. These are large extracellular, plate-like crystals with notched edges. They indicate an inflammatory process.
Synovial fluid
A small amount of hyaluronidase should be added to synovial fluid prior to performing cell counts or preparing cytocentrifuge slides to liquefy the fluid.

Normal cells in synovial fluid include lymphocytes, macrophages, and synovial cells. Neutrophils are often seen since inflammation or infection is a common cause of the effusion. Synovial cells are the lining cells of the synovium, the sac around the joint. These cells resemble mesothelial cells in that they are large with well-defined oval nuclei. The lining cells usually are not as abundant in synovial fluid as serous fluid. It is not unusual to see cartilage cells in these fluids. Synovial fluid is often sent to the lab to evaluate for crystals. Cholesterol crystals are indicative of inflammation. They are large, extracellular, plate-like, colorless crystals with notched edges.

Clinically significant crystals include calcium pyrophosphate crystals that are seen in pseudogout and monosodium urate crystals that are seen in gout. The morphology of the crystals is helpful in identifying the category of the crystals and polarizing with red compensation will provide definitive information. Calcium pyrophosphate crystals are colorless, intracellular crystals that may be rhomboid, square, or thin and elongated. They often look like intracellular crushed glass and there may be abundant crystals in a single cell. Mono-
sodium urate crystals are colorless, needle-like crystals that may be intracellular or extracellular. When these crystals are viewed under polarization with red compensation, calcium pyrophosphate crystals appear blue when the long side of the crystal is parallel to the slow or y-axis and monosodium urate crystals appear yellow when the long side of the crystal is parallel to the y-axis. See Figures 5 and 6.

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WORKS CITED
ARDS = adult respiratory distress syndrome; BAL = bronchoalveolar lavage; LE = Lupus Erythematosus.

INDEX TERMS: body fluids; cerebrospinal; serous; synovial.


LEARNING OBJECTIVES
1. Describe the significance of eosinophils in urine.
2. Describe lupus erythematosus cells in serous and synovial fluids.
3. Describe the significant cellular findings in bronchial alveolar lavage (BAL) specimens.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, Memphis TN.

Address for correspondence: Leilani Collins MS MT(ASCP)SH CLS(NCA), associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, 930 Madison Avenue, Suite 670, Memphis TN 38163. (901) 448-6299. lcollins@utmem.edu.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is the Focus: Body Fluids guest editor.

Cytocentrifuge slides can provide important information in diagnosing various conditions.

Urine for eosinophils
Urine samples for eosinophils may be submitted when inter-

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stitional nephritis secondary to antibiotic therapy is suspected. Slides to search for eosinophils should be prepared by concentration of the sample by either standard centrifugation or cytocentrifugation. Slides should be stained with Wright stain and a thorough search made for eosinophils. A finding of any eosinophils is significant.

Lupus Erythematosus (LE) cells
LE cells are neutrophils containing a smooth homogeneous nuclear mass. The nucleus of the neutrophil will be displaced to the edge of the cell wall by the large homogeneous mass. In patients who have systemic lupus erythematosus, the characteristic LE cell may be seen in serous and synovial fluids. All the factors necessary for formation of these cells—incubation, trauma to the cells, and the LE factor—are present in vivo in patients with systemic lupus erythematosus. The finding of these cells can be of diagnostic significance in patients not previously identified with this disease. See Figure 1.

Bronchoalveolar lavage (BAL)—the fluid that isn’t a fluid
Bronchoalveolar lavage (BAL) is usually performed on hospitalized patients who have non-resolving pneumonia in an attempt to identify organisms that are not responding to antibiotic treatment or to detect malignancy. The “fluid” is obtained by introducing warmed saline into the lungs through

Figure 1. Two LE cells in synovial fluid
FOCUS: BODY FLUIDS

a bronchoscope in 20 mL aliquots followed by aspiration of the saline. A total of 100-120 mL of saline is introduced and aspirated with a recovery of 40-60 mL. The “fluid” is sent to the laboratory for extensive microbiology testing and cytology examination. Occasionally, cell counts and differential counts are requested. Any laboratory performing these procedures needs to be aware that organisms in these specimens are airborne and caution should be taken when containers of fluid are mixed and opened. Containers should be opened under a biological safety hood, tests should be “set up” under the hood (i.e., hemacytometers charged, cytofunnels filled, films prepared), and laboratory personnel performing these procedures should wear a mask while performing cell counts. There are no reference ranges for cell counts on these “fluids” and they have no clinical significance. As in any fluid, the types of cells present are significant. In BAL, the most clinically meaningful information is supplied by the extensive microbiological testing for bacteria and fungus that is the purpose for performing the procedure. The presence of numerous neutrophils can indicate infection or inflammation; numerous eosinophils can indicate allergic reactions, parasitic infections, or eosinophilic pneumonia; and excessive lymphocytes can indicate conditions including viral pneumonia, connective tissue disorders, or lymphoma. Macrophages containing tar can be seen in samples from patients who smoke. Erythrophages may be seen if there has been hemorrhage in the lungs more than 12 hours prior to the procedure. Ciliated epithelial cells are columnar epithelial cells found in the upper respiratory tract. They are elongated cells with the nucleus on one end of the cell and cilia on the other.

See Figure 2. If seen on the hemacytometer when performing cell counts on fresh specimens, these cells appear mobile as they are propelled by their cilia. Type II pneumocytes, resembling adenocarcinoma, can be seen in patients with adult respiratory distress syndrome (ARDS). Type II pneumocytes are very large, dark-staining cells in clumps containing large vacuoles that contain surfactant. Bacteria engulfed by neutrophils may be seen in cases of bacterial infection. Pneumocystis organisms, if present, will appear as amorphous material that, upon careful examination, may contain the round cysts characteristic of the organism. See Figure 3.

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WORKS CITED


Figure 2. Ciliated epithelial cells in BAL specimen

Note nucleus at one end of cell, elongated cytoplasm, and cilia at end opposite nucleus.

Figure 3. Pneumocystis in BAL specimen
CONTINUING EDUCATION QUESTIONS

1. In gout, the crystal found in synovial fluid would have the following characteristics:
   a. Large, extracellular plate-like crystals with notched edges
   b. Small, intracellular rhomboid or square crystals that polarize blue on the slow axis when using red compensation
   c. Intra- and extracellular needle-like crystals that polarize yellow on the slow axis when using red compensation
   d. Large extracellular irregular shaped crystals that will appear turquoise when Wright-stained

2. One tube of bloody CSF is received. Which of the following findings would indicate that the blood was the result of “traumatic tap”?
   a. Clear supernatant when an aliquot of the fluid is centrifuged
   b. The presence of ependymal and cartilage cells on the slide preparation
   c. The presence of nucleated red blood cells on the slide preparation
   d. All of the above

3. A slightly bloody CSF specimen from a patient in the ER complaining of neck pain is received in the laboratory. A CBC received earlier on this patient had a WBC of 52.0 x 10^6/L with 85% blast cells on the differential. On the concentrated slide on the CSF, several RBCs and 87% blast cells are found. How should these results be reported?
   a. The number of blast cells should be reported as seen.
   b. The number of blast cells in the peripheral blood should be subtracted from the number of blasts in the CSF and the blasts in the CSF reported as two percent.
FOCUS: BODY FLUIDS

c. A request should be made that a repeat specimen be submitted in two to three days and report that peripheral blood contamination is present.

4. The lining cells found in serous fluids are:
   a. mesothelial cells.
   b. synovial cells.
   c. ependymal cells.
   d. choroid plexus cells.

5. Exudative effusions are seen in:
   a. malignancies, infections, trauma.
   b. chronic heart failure, cirrhosis.
   c. intestinal obstruction.
   d. meningitis.

6. Pseudochylous effusions may contain:
   a. monosodium urate crystals.
   b. cholesterol crystals.
   c. calcium pyrophosphate crystals.

7. The finding of eosinophils in urine is indicative of:
   a. pyelonephritis.
   b. parasitic infection.
   c. interstitial nephritis.
   d. cystitis.

8. Lupus erythematosus cells may be seen in:
   a. serous fluid.
   b. synovial fluid.
   c. cerebrospinal fluid.
   d. a and b only.

9. When performing a cell count on a body fluid, a clear fluid indicates:
   a. the nucleated cell count is <200/mm³.
   b. the nucleated cell count is <20/mm³.
   c. the nucleated cell count is >200/mm³.
   d. there are no red cells present.

10. A body fluid specimen has a nucleated cell count of 853/mm³. What saline dilution would produce a monolayer cytocentrifuge slide?
    a. 1:2
    b. 1:6
    c. 1:8
    d. 1:12

11. A CSF on a patient complaining of headache is received in the laboratory. The specimen appears cloudy. The nucleated cell count is 434/mm³. On the cytocentrifuge slide there are 90% lymphocytes demonstrating pleomorphism. The most likely diagnosis on this patient is:
   a. cryptococcal meningitis.
   b. viral meningitis.
   c. bacterial meningitis.
   d. lymphoma.

12. Four tubes of CSF from an unconscious patient are received in the lab. All four tubes are equally bloody and when an aliquot of fluid is centrifuged, the supernatant is xanthochromic. On the cytocentrifuge slide, several erythrophages and siderophages are seen. The cause of the bloody fluid is:
   a. traumatic tap.
   b. leukemia.
   c. low platelet count.
   d. subarachnoid hemorrhage.

13. Cells that may be seen in normal CSF include all the following except:
   a. lymphocytes.
   b. monocytes.
   c. neutrophils.

14. A pericardial fluid was received in the laboratory. The appearance of the fluid was pale yellow and clear. The specific gravity of the fluid was 1.012, the protein was 2.1 g/dL, and the LDH was 150 IU. The nucleated cell count was 456/mm³ and the differential was 32% neutrophils, 41% lymphocytes, and 27% macrophages. This fluid would be classified as:
    a. an effusion.
    b. an exudates.
    c. a transudate.

15. Which cell morphotype is predominant in the CSF of the neonate?
   a. Monocyte
   b. Lymphocyte
   c. Neutrophil
   d. Nucleated RBC

16. When the appearance of a serous fluid is bloody, the fluid can be classified as a(n):
    a. transudate.
    b. traumatic tap.
FOCUS: BODY FLUIDS

c. exudate.

d. effusion.

17. On a cytocentrifuge slide on a peritoneal fluid, several large cells were seen, some in clumps and clusters. These cells demonstrated basophilic staining and some were multinucleated and had cytoplasmic vacuoles around the edges of the cells. All nuclei had well-defined nuclear envelopes. These cells should be classified as:

a. malignant.
b. mesothelial.
c. macrophages.
d. lupus erythematosus cells.

18. The presence of eosinophils in CSF is indicative of:

a. bacterial meningitis.
b. malignancies.
c. viral meningitis.
d. allergic response to shunt material.

19. Bronchoalveolar lavage is performed:

a. to determine the cause of persistent pneumonia in hospitalized patients.
b. to remove infectious organisms from the lungs.
c. to determine the origin of metastatic carcinoma.
d. to assess the extent of lung damage secondary to nicotine inhalation.

20. When three tubes of spinal fluid are received in the laboratory, tube #1 should be used for:

a. chemical testing.
b. hematology testing.
c. microbiology testing.
d. cytology.

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10. a b c d  20. a b c d
CONTINUING EDUCATION

Answers to 2007 FOCUS
Continuing Education Questions

THROMBOCYTOPENIA
9. The autosomal dominant abnormality associated with decreased platelet production is:
   a. May-Hegglin anomaly.
   b. Ristocetin
10. Which of the following is not a hallmark of ITP?
    a. Megakaryocyte hypoplasia
    b. Stop heparin infusion immediately
11. What is the first step in treatment of HIT?
    a. Aspirin
12. A defect in primary hemostasis (platelet response to an injury) often results in:
    a. A defect in secondary hemostasis
    b. Mucosal bleeding.
13. When a drug acts as a hapten to induce thrombocytopenia, an antibody forms against:
    a. The combination of the drug bound to a platelet membrane protein.
14. TAR refers to:
    a. Abnormal bone formation including hypoplasia of the forearms.
15. HUS in children is related to:
    a. Diarrhea secondary to Shigella sp.

QUALITATIVE PLATELET DISORDERS
16. A defect in GP IIb/IIIa causes:
    a. Glanzmann thrombasthenia.
    b. Essential thrombocytopenia.
17. Aspirin ingestion blocks the synthesis of:
    a. Thromboxane A2.
18. Patients with Bernard-Soulier syndrome have which of the following laboratory test findings?
    a. Abnormal platelet response to ristocetin
19. Which of the following is the most common of the hereditary platelet function defects?
    a. Essential thrombocytopenia.
CONTINUING EDUCATION

20. A mechanism of antiplatelet drugs targeting GP IIb/IIIa function is:
   c. direct binding to GP IIb/IIIa.

21. Which is true of the impaired platelet function in myeloproliferative disorders?
   d. Decreased number of α and dense granules

22. Which is a congenital qualitative platelet disorder?
   b. Ehlers-Danlos syndrome OR
   c. Henoch-Schonlein purpura

23. In uremia, platelet function is impaired by higher than normal levels of:
   d guanidinosuccinic acid.

24. The platelet defect associated with increased paraproteins is:
   a. impaired membrane activation owing to protein coating.
   b. impaired membrane activation owing to protein coating.

20(2) Spring 2007
FOCUS: IMMUNOHEMATOLOGY
APPLYING QUALITY IMPROVEMENT TOOLS IN THE TRANSFUSION SERVICE
1. Which of the following QA tools can be used for root cause analysis?
   d. Fishbone Diagram

2. Which of the following QA Tools is used to monitor progress?
   b. Pareto chart

3. The goal of a risk analysis is to:
   c. reduce the risks as much as possible.

4. The _____ is a tool than can be modified to document decisions, monitor project progress or display information.
   a. grid

5. RPN defines the:
   a. amount of risk associated with a failure.

ORGANIZING THE ANTIBODY IDENTIFICATION PROCESS
6. The major sections of the antibody identification form include all of the following areas except:
   b. physician approval.

7. A major advantage of the history section of the antibody identification summary form is that it:
   c. facilitates prioritization of patient work-up.

8. The primary problem associated with discovery of complex antibody specificity in a pre-surgical work-up is:
   a. potential delay of surgery.

9. The primary advantage to using an antibody identification summary form is that it:
   d. standardizes the process.

20(3) Summer 2007
FOCUS: SEMINAL PLASMA
SEMEN ANALYSIS
1. Approximately what percent of U.S. couples experience infertility?
   b. 7-12%

2. Prostatic fluid:
   d. provides enzymes for liquefaction of the coagulum.

3. Semen is a combination of sperm and glandular secretions, primarily prostatic and seminal vesicle fluids.
   d. Seminal vesicle fluid is alkaline, rich in flavin that is responsible for semen color and contributes 70% of the semen volume.

4. The development of flagellated spermatozoa from round cell spermatids is:
   d. spermiogenesis.

5. Patients are advised to abstain from any type of ejaculation for 3 days (2 to 5 days) prior to the semen analysis because:
   c. immature sperm are more likely to be found in the ejaculates following a short abstinence period (< 1 day).

6. An abnormal semen pH is associated with:
   b. disorders of the prostate or seminal vesicles.

7. Mr. X arrived at the andrology laboratory 40 minutes late for his scheduled appointment. He apologized to the technologist processing his semen specimen, and explained that he had been delayed by heavy traffic in the area. The specimen had been collected 80 minutes previously. The technologist was surprised to note a well
formed coagulum and checked the patient information form to determine:
c. if any portion of the ejaculate had been lost.

8. A semen specimen with a brownish color:
b. indicates the presence of old blood indicative of infection, trauma or malignancy.

9. When assessing sperm motility, four categories of sperm motility are scored based on their speed of progression. Rapid progressive sperm have a speed of progression that is ≥ 25 µm /s at 37°C, which can be approximated because 25 µm is equal to:
a. one-half a sperm tail length.

10. The technologist assessing Mr. Z’s motility prepared duplicate slides and rated and categorized the sperm on the first slide as: 201 rapid progressive; 86 sluggish progressive; 0 non-progressive; and 198 immotile and those on the duplicate slide as: 210 rapid progressive; 98 sluggish progressive; 5 non-progressive; and 80 immotile. Is the difference between the duplicates acceptable for reporting?
d. Yes, the difference between duplicates is less than expected for counting error alone.

11. Mary read two properly prepared slides for assessment of motility. She counted a total of 240 sperm on the first slide and categorized according to rate of motility: 103 rapid progressive, 20 sluggish progressive, 0 non-progressive, and 117 immotile. On the second slide she counted 293 sperm categorized as: 150 rapid progressive, 29 sluggish progressive, 1 non-progressive, and 113 immotile. Is the difference between the duplicates within the acceptable 95% confidence interval?
b. No, the difference between the duplicates cannot be greater than 34.6.

12. Vitality staining is performed to determine if immotile sperm are alive or dead. The Eosin stain or the Eosin-nigrosin stain may be used to make this determination. Sperm that are alive or dead will appear differently depending on the staining procedure. When performing the vitality stain:
b. dead sperm stained with Eosin-nigrosin will appear red against a dark background.

d. Agglutination:

13. Agglutination:
d. refers to the specific attachment of motile sperm to each other.

14. Spermatozoa were counted in 5 large squares (refers to 5 of the 25 squares within the Central square) on a Neubauer hemacytometer. Average number of sperm from the chamber count = 66; dilution 1 in 20. Each of the 25 squares is 0.04mm². The sperm concentration in millions/mL is:
c. 66 X 10⁶.

15. The following semen parameters were recorded: volume = 2.0 mL; pH = 7.2; motility = 56%; vitality = 62%; concentration = 50 million/mL. The total sperm count would be:
c. 100 X 10⁶.

16. Please refer to Figure 3. To determine the concentration of sperm, a minimum of 200 spermatozoa should be counted in the central grid on each side of the Neubauer hemacytometer, counting a total of five squares within the grid. Approximately 200 sperm in the five squares are equal to _______cells/ml.
c. 10

17. If a concentration of 200 X 10⁶/ml was estimated based on counting 200 spermatozoa/field on the initial assessment, the dilution factor would be made:
c. 1 in 20.

18. If an average of 240 spermatozoa were counted on the Neubauer hemacytometer from a 1 in 5 dilution, the calculated sperm concentration in sperm/ml should be:
d. 62.5X 10⁶.

19. To be considered normal a sperm must meet strict criteria for both shape and size, which is determined by measuring with a calibrated ocular micrometer. The following sperm, stained with the Papanicolaou stain would be classified as normal:
b. Oval in shape, 4.5 µm X 3.5 µm; acrosome covering 40% of the anterior head; tail length 45 µm

20. Cytoplasmic droplets:
d. stain green with the Papanicolaou stain.
21. The teratozoospermia index (TZI):
   a. is calculated by dividing the total number of defects by the total number of abnormal sperm.

22. Round cells(‘):
   b. may be leucocytes that should not total more than 1 million/mL semen

23. The reference range for the strict criteria sperm morphology is:
   c. > 14% is currently considered to be the threshold for assisted reproduction.

24. Proficiency testing for semen analysis is:
   a. available from College of American Pathologists and the American Association for Bioanalysts.

6. A patient suffering from a deficiency of the enzyme responsible for cleaving ultra-large von Willebrand factor molecules would best be treated by:
   c. daily plasmapheresis until the platelet count returns to normal.

IS THERE A GENETIC RELATIONSHIP BETWEEN ARTERIAL AND VENOUS THROMBOSIS?

7. Venous thrombosis is generally associated with:
   b. pulmonary embolism.

8. Arterial thrombosis is traditionally characterized by all of the following EXCEPT:
   c. changes in blood composition (thrombophilia).

9. Which of the following supports the concept that arterial thrombosis and venous thrombosis are linked pathophysiologic processes?
   a. Individuals experiencing an idiopathic venous thromboembolic event are more likely to suffer a significant arterial event than the general population.

CROSS TALK BETWEEN THE INFLAMMATION AND COAGULATION SYSTEMS

10. Which of the following cells is NOT considered a significant contributor to inflammation?
    a. Lymphocytes

11. Which of the following best describes the function of the inflammatory response?
    b. To neutralize or eliminate pathogens (or to destroy injured or necrotic tissue) as part of the host defense

12. All of the following are considered important inflammatory cytokines EXCEPT:
    d. Interleukin-3 (IL-3).

13. The effect of inflammation on the protein C anticoagulant system is to:
    a. decrease thrombomodulin.

14. Which of the following statements concerning the effect of inflammation on the hemostatic system is correct?
    c. Increases procoagulant activity of the endothelium

15. Thrombin and other coagulation proteases activate cells via binding to PAR receptors. PAR stands for:
    d. protease activated receptor.
16. The major effect of the procoagulant inhibitors, anti-thrombin (AT), activated protein C (APC), and tissue factor pathway inhibitor (TFPI) on inflammation is to:
   a. decrease the production of inflammatory mediators and leukocyte activation.
   b. inactivate C3a and C5a.

17. The role of thrombin activated fibrinolysis inhibitor (TAFI) in inflammation is to:
   b. inactivate C3a and C5a.

**FOCUS: PROTEOMICS**

**INTRODUCTION TO PROTEOMICS**

1. The scientific approach to analyze all proteins expressed by a genome is known as:
   c. proteomics.

2. The main goal of proteomics is to:
   d. all of the above.

3. Objectives of clinical proteomics are all of the following EXCEPT:
   b. identify processes of protein expression.

4. In certain genes, the combination of exons can make a gene become active and each combination may result in a different protein. This process is known as:
   a. alternative splicing.

5. Eukaryotic gene expression and regulation at the transcriptional level occur by all of the following EXCEPT:
   d. chemical alteration.

6. Post-transcriptional modifications of proteins occur by all of the following EXCEPT:
   c. polyadenylation.

7. Expression proteomics differs from functional proteomics in that:
   c. expression proteomics compares the expression profiles of protein patterns.

8. Which of the following techniques has been considered for the past 20 years the standard technique for analyzing proteins?
   b. Two-dimensional electrophoresis

9. The chronological order for identification of proteins using 2-DE is:
   d. protein separation by isoelectric point followed by separation by molecular weight followed by mass spectrometry.

10. The mass spectrometry techniques used for the identification of proteins are all the following EXCEPT:
    b. Liquid Crystal Multiple Sequence Mass Spectrometry (LC-MS-MS).

**PROTEOMICS TECHNOLOGY**

11. The most important factor during protein sample preparation is:
    b. protein solubilization.

12. The main goal of protein sample preparation is to:
    c. increase protein solubility, reduce complexity, and eliminate nucleic acid.

13. Methods used to reduce protein complexity are all of the following EXCEPT:
    d. pre-fractionation.

14. The mass spectrometry techniques used for the identification of proteins include all of the following EXCEPT:
    b. Electromagnetic Ionization – MS/MS (EM-MS).

15. All of the following are true concerning first dimension 2-DE EXCEPT:
    a. cup loading of the sample should be used to decrease production of artifacts.

16. The five chronological steps in a classical 2-DE analysis are:
    b. normalization, protein spot detection, matchset generation, quantitation of protein spots, and statistical analysis.

17. The chronological order of the three basic steps involved in mass spectrometry is:
    d. ionization of protein molecules, mass-to-charge ratio separation, and analyzer detection.

18. What is the major difference in protein analysis between MALDI and ESI?
   a. MALDI protein analysis occurs in solid phase and ESI analysis occurs in liquid phase.
19. All of the following are true concerning ICAT EXCEPT:
   c. that it is unable to quantitate proteins from a complex mixture.

20. In second dimension 2-DE all proteins previously separated via first dimension 2-DE are subsequently separated according to their molecular weight. This is accomplished by using:
   b. different concentrations of acrylamide gels.

PROTEOMICS: CLINICAL APPLICATIONS
21. Clinical proteomics offers the opportunity to:
   d. all of the above.

22. Which of the following is the most common technique used in proteomics technology?
   d. Two-dimensional electrophoresis

23. All of the following are challenges to proteomic clinical application except:
   b. analysis of bacterial virulent strains of protein expression.

24. Proteomic technology includes which of the following?
   d. a, b, and c

25. Many tumor markers lack specificity and sensitivity. In contrast, the use of proteomics applications in diagnosis and prognosis of cancer offers a more reliable biomarker by detecting:
   b. aberrant proteins.

26. Alpha-beta crystallin and tropomyosin are examples of clinical proteomic application correlates with:
   a. cardiac allograft rejection.

27. Proteomics methodology in microbiology can be simplified by the use of:
   c. bacterial protein enriched fractions.

28. This question was not graded.
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GET RESULTS
NATIONAL MEDICAL LABORATORY PROFESSIONALS WEEK

April 19-25, 2009

During National Medical Laboratory Professionals Week, April 19-25, 2009, we’re celebrating the people and practices that keep the focus on quality care. It is a demonstration of commitment we’re carrying to communities across the nation—and a spotlight on the professionals who provide outstanding results every day of the year.

United by our commitment, we’re coming together this April under the national theme “Laboratory Professionals Get Results.” It’s a pride-instilling phrase paying tribute to the dedicated men and women who set a standard of excellence throughout the year. Join us as we celebrate the achievements of this essential segment of the health care community.

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