Effecting Translational Research Practices

ISAAC D MONTOYA

The issue of transferring medical research findings into practice is currently a topic of concern in most scientific circles. Our society has never experienced such an explosion of new scientific findings as we have seen in recent years. Yet practitioners/clinicians do not adopt many of these new findings into their practice. Scientists and research funding organizations such as the National Institutes of Health (NIH) remain frustrated over this issue termed ‘translational research’. For example, the budget of the NIH last year was $24 billion for medical research. That is a large portion of the Federal Government’s budget and represents the hard earned tax dollars of U.S. citizens. This investment in medical research yielded many new discoveries yet they remain largely in scientific journals. They have yet to be incorporated into practice and do not benefit the society that paid for them. The reasons for this are varied. Some studies on the problem cite the large masses of new information practitioners have to continually absorb. Others cite human nature and its resistance to change.

The clinical laboratory is not immune from this issue. New procedures are constantly being developed. The question begs which procedures are to be added to the formulary of laboratory procedures. If all new procedures were added to every laboratory, our laboratories would be very large, expensive, and inefficient. The model that the laboratory profession has chosen to follow over many years is having reference laboratories provide the newer, more esoteric tests and the local laboratories provide the more routine and frequently ordered tests. As the esoteric tests transition to a routine nature, the local laboratories incorporate them into their formulary. Other factors come into play in this process such as the specialty type of physician using a particular laboratory and what laboratory tests they require to augment their clinical decisions. So in one sense the process is better developed than in other professions.

One must also examine who conducts the research, which is what advances any scientific discipline or field. This question is particularly pertinent to clinical laboratory science (CLS). Who advances the field of CLS? Who advances the field in any discipline? If we look at the basic sciences, we see biologists conducting research in biology, chemists conducting research in chemistry, and physicists conducting research in physics. In the social sciences we see psychologists conducting research in psychology, sociologists conducting research in sociology, and anthropologists conducting research in anthropology. However in healthcare there is more of an interdisciplinary approach. A team of physicians, nurses, CLSs, pharmacists, and other healthcare professionals often conducts research regarding prevention or treatment of a disease. Yet within each of these domains there is a certain amount of research that is specific to that domain and conducted by that profession.

Historically the field of CLS has been advanced by other disciplines and not CLS. For example, clinical chemists research promising methods for detection of specific substances using chemical reactions. Microbiologists also investigate new methods of identifying specific bacteria, viruses, and parasites. Oncologists have done most of the work in researching hematologic advances. Companies that manufacture laboratory equipment then capitalize on these research findings. They take the science and couple it with their engineers to commercialize the process. The commercialization may result in a simplified prepackaged manual process or in an automated piece of equipment. The CLS then learns how to use the equipment and possibly conducts some in-service on the new test to other healthcare professionals. So is the involvement of the CLS in advancing the field? Very little, if any. Is this situation a barrier or a protective factor in promoting the profession and serving patients? One perspective is that it is a positive situation as the PhD prepared chemist or microbiologist is better prepared to conduct research and advance the field while the CLS is best at simply running the tests without thought or creativity. Another perspective is that CLSs know the day to day needs of their
patients and clinicians and they should be the ones working in concert with other researchers to advance the field of CLS.

If the model used by other disciplines is followed, then the CLS profession should be actively involved in developing new scientific discoveries and advancing these findings to the clinical laboratory. One may argue that CLSs are involved with the companies that sell laboratory equipment. This is true; however, their involvement is typically in the preparation of instructional material, marketing, and technical service, not in the research/developmental aspect. These services are needed and important but they do not per se advance the field in the manner that occurs in other disciplines. So what is needed? Perhaps it is time to rethink the field of CLS. Is it simply one of running tests in the laboratory and spitting out results or is it one that is a true practitioner that is involved in developing its own field and applying the knowledge it has gained to improve the healthcare of the people it serves?

I would argue that laboratory medicine has become very sophisticated and complex. Not necessarily in the performance of tests but inclusive of the continuum from new scientific discoveries, specimen processing and testing, to the management of laboratory data that optimizes laboratory services. We find ourselves in the same situation as nursing and pharmacy. Those professions have also experienced an explosion of new knowledge and the practice of their professions has become quite sophisticated out of necessity. Nursing has addressed the problem by establishing numerous doctorate programs in nursing and convincing Congress to establish the National Institute of Nursing Research (NINR). NINR is part of the National Institutes of Health. Its sole purpose is to conduct research in nursing so as to advance the field of nursing. Pharmacy has gone a step further and now requires the doctorate degree as the entry-level degree to the profession. The doctorate in pharmacy (Pharm D) is a professional degree designed to prepare an individual to practice the new pharmacy model which includes patient assessment, counseling, and medication management as well as filling of prescriptions. Both nursing and pharmacy offer professional degrees as well as the PhD that prepares an individual to conduct research. Both of these models have strengths and limitations. Will these models work for the clinical laboratory? The professional doctorate degree may serve the CLS profession well; however, a generic PhD in CLS may not be able to compete with the PhD in chemistry or microbiology for the simple reason that the PhD in CLS would have to master chemistry, microbiology, virology, hematology, immunology, and the other laboratory specialties. This is not realistic, requiring the need for a new model. Perhaps the model used in pharmacy offers some potential. Pharmacy offers a joint degree program consisting of the Pharm D and PhD. The PhD in CLS could specialize in one of the subspecialties of the laboratory, e.g., chemistry, microbiology, or immunology. Progression in any profession occurs in step wise fashion. The development of programs such as these are the logical extension of the field as we engage in quality patient care on the floors and in the research laboratory by providing a professional who has both the clinical skills and research skills necessary to work as a researcher.

Few would argue that automation in the laboratory has made testing straightforward and the need for highly educated and trained personnel is not necessary to simply do testing. What many have failed to recognize is the big picture that encompasses all aspects of laboratory medicine not just the testing phase. This lack of perspective limits the benefits of the laboratory to both patients and clinicians. Another reality is that someone from outside the profession is not going to make this happen. Not administrators, third parties, or the government. Whatever the models used to accommodate the complexity and sophistication of contemporary laboratory medicine must stem from the professionals themselves. It won’t be easy to agree amongst the profession but a realistic compromise that advances the field to the benefit of the patient and the profession has to be reached. This model must then be marketed to other healthcare professionals, third party payers, and above all the public. It will not be easy but it hasn’t been easy for any other profession. It is part of the price to pay when advancing the field.

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Washington Beat is intended to provide a timely synopsis of activity in the nation's capital of importance to clinical laboratory practitioners. This section is coordinated jointly by Kathy Hansen, Chair of the ASCLS Government Affairs Committee, and Don Lavanty, ASCLS Legislative Counsel. Direct all inquiries to ASCLS (301) 657-2768 extension 3022; (301) 657-2909 (fax); or mail to ASCLS, 6701 Democracy Blvd., Suite 300, Bethesda MD 20814, Attention: Washington Beat.

The American Society for Clinical Laboratory Science (ASCLS) has worked for state licensure of laboratory personnel for many years. This has been a challenge, and it has been a number of years since a state was successful in passing a state licensure bill. The most recent success was Montana in 1993. Despite the challenges, a number of constituent societies of ASCLS have continued in their efforts toward licensure, and have attempted to build coalitions of laboratory professional organizations to support their efforts. In every case, however, it has been the ASCLS-based group who was the leader in licensure efforts.

At the ASCLS national meeting, a “State Licensure and Legislative Update” session is held annually. The August 2003 session was a panel presentation that had three purposes: to present updates from states that are in various stages of trying to pass licensure legislation, to review post-implementation issues that are currently arising in already licensed states, and to review other state initiatives related to laboratory personnel.

Interest in personnel licensure has been heightened in the past two years due to:

- the need for better data on numbers of practitioners, related to bioterrorism readiness.
- worsening laboratory personnel shortages, tied to the need for recognition of the profession.
- public concern about medical errors and patient safety.

The purpose of licensure is to protect:

- public health and safety.
- laboratory scope of practice by excluding those who have not acquired appropriate requisites for licensure.

Certainly both of these purposes are consistent with the current emphasis on patient safety and healthcare provider competence. Other advantages are improvement of the quality of testing, respect and prestige for laboratorians, increased visibility to the public, and definition of scope of practice.

Gilma Roncancio-Weemer of Illinois reported on efforts to pass a licensure bill there. ASCLS-IL has worked since 1999 to build a coalition of support and to draft bill language. The bill includes standards for licensure of clinical laboratory scientist (CLS) and categorical clinical laboratory technician (CLT) levels, but does not include histotechnicians, pathology assistants, or phlebotomists. Initial licensure requires passing a recognized national certification examination and re-licensure requires documented continuing education. An important feature is ‘grandfathering in’ of existing practitioners, so that there should be no concern about laboratorians losing jobs.

Illinois Senate Bill 1068 was introduced and sent to a committee for hearings. Written support was obtained from ASCLS-Illinois, the American Association for Clinical Chemistry (AACC)-Chicago, the American Society for Clinical Pathology (ASCP), the Illinois State Society of American Medical Technologists (ISSAMT), and the Illinois Society of Microbiology (ISM), with participation from the Clinical Laboratory Management Association (CLMA). The American Association of Bioanalysts (AAB) opposed the bill. In addition, there was opposition from the Illinois Society of Pathology, the Illinois Hospital Association, and the state medical society. Some of the stated grounds for opposition reflected lack of knowledge about the provisions of the bill: concern about current laboratorians losing jobs, what duties CLTs could perform, etc.

Future steps will include more education and bridge-building to the other interested organizations, and continued efforts to educate the public, other healthcare professionals, and government officials about the need for regulation.

Rick Panning of Minnesota reported on the efforts during the past year to build a coalition of support and educate laboratorians in the state about licensure. Rick and others have
traveled the state to present informational sessions and judge the extent of support from the laboratory community. In addition to a long list of professional associations, meetings have been held with the Minnesota Department of Health and the Mayo Clinic to share information and garner support.

Future steps will include asking professional associations for formal commitment to support the effort, continuing to improve understanding of the legislative process in the state, beginning to draft bill language, and looking for sponsors in the Minnesota House and Senate.

Leticia San Diego of Michigan reported that two bills of interest were introduced into the Michigan legislature this spring. House Bill 4554 was introduced to amend the state's licensure bill to add licensure of laboratory personnel (CLS, CLT, and clinical laboratory assistant) to the list of other healthcare professions that are licensed in the state. The interest from the state legislature is tied to its concerns about bioterrorism readiness. The Michigan Society for Clinical Laboratory Sciences (M SCLS) is in the process of contacting other laboratory professional associations for their support, and attempting to counter any opposition.

The second bill in Michigan is SB 4272, “The Creation of the Governor’s Commission on Patient Safety”. It establishes a commission that would conduct public hearings and solicit input from the public and from healthcare organizations that have interest in patient safety. The commission is charged with making a report to the legislature with recommendations for error reduction and improvement of medical practice.

Dana Duzan of Washington State reported on the work of the Washington Health Care Personnel Shortage Task Force. Dana represented all of allied health on the task force, which included educators, healthcare administrators, unions, professional associations, etc.

The charge to the task force was to:
- identify ways to improve student recruitment and retention, marketing, and outreach into health careers, including ways to increase the diversity of health professions.
- recommend modifications to state regulations and statutes to help alleviate the shortage.

The task force subcommittees did extensive research and developed strategies. They produced a comprehensive report that is available at www.wtb.wa.gov/HEALTHCARE TASKFORCE.HTM. It contains excellent information that could be of use to other states also.

Helen Bixenman of Arizona, current president of the National Credentialing Agency (NCA) reported on recent developments in California regarding application of licensure laws. California has implemented phlebotomy licensure, which requires passage of one of the national certification examinations recognized by the state. NCA has applied to be one of the recognized agencies. California will soon implement licensure of CLTs for the first time, and plans to use national certification exams for that licensure as well.

California has recognized the NCA specialty examinations for Cytogenetics and Molecular Biology for a number of years, but recently began enforcement of licensure for those practitioners. That necessitated a flurry of activity for those individuals and the NCA office as people needed to produce proof of their NCA certification.

California did not administer a CLS examination in the spring of 2003 due to budget limitations, and has issued temporary licenses to individuals who qualify to take the California exam. Since the pass rate for the California exam is quite low, there is concern about whether unqualified individuals are now practicing.

ASCLS remains committed to licensure for laboratory personnel. The reports from the panelists illustrate that even once licensure is in place, there are still changes to the process and threats to scope of practice. As professionals, we must stand ready to protect our patients’ safety and our professional scope of practice.
Methicillin resistant strains of Staphylococcus aureus (MRSA) are implicated in serious infections and nosocomial outbreaks, and show resistance to a wide range of antibiotics, thus limiting the treatment options. Therefore, rapid detection is clinically crucial for both treatment and infection control measures. This study assessed the performance of a rapid latex agglutination kit marketed to detect MRSA clinical isolates (MRSA-Screen test Denka Seiken Co Ltd, Tokyo, Japan) based on detecting a specific penicillin binding protein 2a (PBP2a) in comparison to the NCCLS oxacillin salt agar screen plate, the 1µg oxacillin disk diffusion test, and the oxacillin MIC by E-test. Testing was carried out on 133 isolates consisting of 99 MRSA and 34 methicillin sensitive strains of S. aureus (MSSA).

Concordant results were observed between the latex kit and all the other tests for the 99 MRSA isolates. Only 1 of the 34 MSSA isolates gave a positive agglutination reaction in the latex kit. The kit sensitivity and specificity were determined to be 100% and 97%, respectively. This reliable performance indicates that the MRSA-Screen latex test is a very useful test for the rapid detection of MRSA isolates in the clinical microbiology laboratory.

**ABBREVIATIONS:**
- CNS = coagulase negative staphylococci
- MIC = minimum inhibitory concentration
- MRSA = methicillin resistant S. aureus
- MSSA = methicillin susceptible S. aureus

**INDEX TERMS:** latex kit; methicillin resistance; rapid detection; Staphylococcus aureus

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The increasing global encounter of methicillin resistant Staphylococcus aureus (MRSA) strains is causing a wide spectrum of hospital- and community-acquired infections. Moreover, the limitation incurred upon treatment options has been inflicting a substantial toll of morbidity and mortality. Thus, rapid detection of MRSA strains is essential for proper treatment and specific infection control measures. The conventional techniques of oxacillin disk diffusion, agar screen, and minimum inhibitory concentration (MIC) determinations take at least 24-hours for the results to be available. Moreover, their accuracy is influenced by several factors including inoculum size, incubation time and temperature, media, pH, and salt concentration. Though molecular testing requires a shorter time, it is technically challenging and expensive.

To overcome the above noted testing problems, a latex agglutination kit (MRSA-Screen test Denka Seiken Co Ltd, Tokyo, Japan) was recently introduced for the rapid detection of MRSA isolates based on the detection of penicillin binding protein 2a (PBP2a) in these isolates. The turnaround time for results by this test is within half an hour of acquiring a fresh isolate.

This study was undertaken to evaluate the performance of this kit in a clinical microbiology setting at a tertiary care medical center, in comparison with the conventional oxacillin disk diffusion and agar screen methods, and MIC determination by the E test.

**MATERIALS AND METHODS**

**Isolates**
A total of 133 consecutive clinical strains of S. aureus isolates representing 99 MRSA and 34 methicillin susceptible S. aureus (MSSA) were tested in this study. These were col-
lected between October 1999 and October 2000, and categorized based on the oxacillin disk and agar screen methods. The isolates were stored in brucella glycerin broth at −20°C until simultaneously tested by the various methods discussed below. Prior to testing, each isolate was subcultured three times on trypticase soy agar containing 5% sheep blood to ensure purity and freshness.

**Identification**
Identification of staphylococcal isolates was based on standard procedures including colony morphology, Gram stain appearance, the catalase reaction, and the tube coagulase test. In addition, the mannitol salt fermentation test and the Slidex Agglutination Test (bioMérieux, France) were used to differentiate *S. aureus* from coagulase negative staphylococci (CNS). Additionally, the API Staph (bioMérieux, France) was used when test results were in doubt or not clear.

**Oxacillin disk diffusion test**
The 1µg oxacillin disk diffusion test, using Mueller-Hinton agar without supplementation with sodium chloride, was performed and interpreted according to the NCCLS guidelines. After inoculation, the plates were incubated at 35°C and results recorded after 24-hours of incubation. *S. aureus* isolates that showed inhibition zone size less than 13 mm or those showing mutants within the inhibition zone were considered resistant (less than or equal to 10 mm), or intermediate resistant (11 mm to 12 mm). Those with zone sizes greater than or equal to 13 mm were considered susceptible.

**Oxacillin salt agar screen plate**
Isolates inoculated on Mueller-Hinton agar plate containing 6 µg/mL oxacillin and 4% NaCl were incubated at 35°C for 24-hours and interpreted according to reported procedure. Growth indicated resistance, and absence of growth indicated susceptibility to methicillin.

**E-test**
The E-test strips (PDM-Epsilometer, AB Biodisk, Solna, Sweden) were used to determine the MICs of oxacillin for the *S. aureus* isolates. The medium used was Mueller-Hinton agar supplemented with 2% NaCl. Inoculated plates were incubated at 35°C for 24-hours, and readings of MICs were determined where the border of the elliptical inhibition zone intersected the scale on the oxacillin test strip. An MIC value of greater than or equal to 4 µg/mL was taken as resistant and an MIC less than or equal to 2 µg/mL was considered susceptible. An MIC value between "greater than 2 µg/mL" and "less than 4 µg/mL" was considered intermediate/borderline.

**MRSA-screen test**
Extraction of penicillin binding protein (known as PBP2a) from fresh isolates, after overnight growth, was carried out according to the following method.

#### Table 1. Overall comparative findings of oxacillin assays versus MRSA-latex assay for the 99 MRSA and 34 MSSA isolates

<table>
<thead>
<tr>
<th>Oxacillin assay (breakpoints)</th>
<th>Number tested</th>
<th>Number of isolates showing MRSA-latex:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Agar plate screen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Susceptible</td>
<td>34</td>
<td>1*</td>
</tr>
<tr>
<td><strong>1 µg disk diffusion zone (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant (&lt;10)</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Intermediate (11-12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible (≥13)</td>
<td>34</td>
<td>1*</td>
</tr>
<tr>
<td><strong>E test (µg/mL)</strong></td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Resistant (≥ 4)</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Intermediate (2.1-3.9)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible (≤ 2)</td>
<td>31</td>
<td>1*</td>
</tr>
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</table>

* Refers to isolate code number 84 (see Table 2 for its detailed susceptibility)
to the manufacturer's instructions (Denka Seiken Co Ltd, Tokyo, Japan). Briefly, 10 to 20 colonies were emulsified in four drops (200 µL) of Extraction Reagent 1, heated at 100 °C for three minutes in a heating block, neutralized after cooling with one drop (50 µL) of Extraction Reagent 2, and centrifuged at 1500g for five minutes. Then, 50 µL of the supernatant was mixed with 25 µL each of test and control latex separately. The mixtures of supernatant and latex on the circled test cards were rotated for 3-, 6-, and 10-minutes and examined for agglutination. A positive detection of MRSA PBP2a yielded agglutination with test latex but not control latex. Negative reaction was determined when no agglutination occurred in either the test or control latex, while indeterminate results were noted when agglutination was observed with the control latex.

Quality control strains
The performance of these tests was monitored using quality control strains: MSSA (ATCC 25923) and an in-house determined MRSA.

Predictive indices
Predictive indices of sensitivity, specificity, positive and negative predictive values, and accuracy were calculated as reported previously.9

RESULTS
See Table 1 for results of the different tests for the 99 M RSA and the 34 M SSA isolates. All the M RSA isolates, as determined resistant by the oxacillin disk and agar screen methods, were uniformly resistant by the E-test (MICs greater than or equal to 4 µg/mL; mean = 108 µg/mL; range = 4 to greater than or equal to 256 µg/mL) and showed positive reactions by the M RSA-Screen latex kit within three minutes of testing.

Among the 34 M SSA isolates determined susceptible by the oxacillin disk and agar screen methods, 31 (91%) had MICs less than or equal to 2 µg/mL while the remaining three isolates showed MICs ranging between 2.1 and 3.9 µg/mL. The distribution of the M RSA-Screen latex kit reactions, within three minutes of testing, for the 34 M SSA isolates showed a clear negative for 27 isolates, a repeatedly strong positive for one isolate, and a weak positive for six isolates. Weak positive reactions were also observed at six and ten minutes rotation for one and three isolates, respectively. No autoagglutination was observed with test isolates and the control latex reagent. The results of the different tests for these M SSA isolates are presented in Table 2.

Table 2. Characteristics of M SSA isolates that showed strong false and weak positive reactions in M RSA-latex assay

<table>
<thead>
<tr>
<th>M RSA-latex</th>
<th>Isolate</th>
<th>Oxacillin tests results at 24-hour incubation</th>
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<tbody>
<tr>
<td></td>
<td>Code</td>
<td>Agar plate</td>
</tr>
<tr>
<td>Strong reaction at three minutes</td>
<td>84</td>
<td>S</td>
</tr>
<tr>
<td>Weak reaction at three minutes</td>
<td>18</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>158</td>
<td>S</td>
</tr>
<tr>
<td>Weak reaction at six minutes</td>
<td>11</td>
<td>S</td>
</tr>
<tr>
<td>Weak reaction at ten minutes</td>
<td>12</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>S</td>
</tr>
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</table>

S = Susceptible
DISCUSSION

In this study, concordant results were observed between the M RSA-Screen latex test and the conventional oxacillin disk diffusion test, agar screen test, and the E-test for the 99 M RSA isolates. Only one of the 34 M SSA isolates gave a discrepant result by showing a positive agglutination reaction in the latex test while being susceptible with the oxacillin tests. Thus, the M RSA-Screen latex kit had a sensitivity of 100% and a specificity of 97% as shown in Table 3. These predictive values fall within the previously reported ranges of 90% to 100% sensitivity and 94% to 100% specificity for this test kit.\(^{7,10-16}\)

Though slide agglutination tests provide rapid results, they are susceptible to false positive and false negative reactions. In this study, a true false positive M RSA-Screen latex kit result, at three minutes rotation, was noted in 1 of 34 (2.9%) M SSA isolates. This isolate however, showed changing susceptibility between 24- and 48-hours incubation in the other tests. At 24-hours the isolate was susceptible by the oxacillin disk and agar screen as well as by the E test MIC value (2 \(\mu\)g/mL). At 48-hours however, the oxacillin disk test showed appearance of mutants in the inhibitory zone, the oxacillin agar plate revealed growth of colonies, and the E test MIC value increased to 3 \(\mu\)g/mL, suggesting that this isolate could fall in the borderline resistance category. False positive M RSA-Screen latex kit results have been reported to be due to several factors including the use of heavy inoculum, underheating during PBP2a extraction, and prolonged reaction time.\(^{10,11,16}\) Weak false positive agglutination reactions, due to the latter reason, were observed in four of our M SSA isolates when the reaction time was extended for 6 and 10 minutes (Table 2). To avoid these weak positive reactions, Mr. Arriott recommended including a positive and negative control in each test run, and noted also that more reproducible results were obtained when a heating block was substituted for a boiling water bath.\(^{11}\)

False negative M RSA-Screen latex kit results were not encountered in this study. Though rare, false negative results have been reported to occur due to overheating during the extraction procedure, indicating that heating is considered a critical step in this assay.\(^{11}\) Moreover, Van Leuwen found negative M RSA-Screen latex testing in the presence of positive mecA PCR.\(^{13}\) To overcome this false negative reaction, they suggested inducing the mecA gene by exposing isolates to methicillin before performing the test. Other recommendations to improve the sensitivity of the assay included increasing the inoculum size, and prolonging the reaction time (up to 15 minutes) to reveal and make the agglutination reactions more pronounced.\(^{7,12,14}\) This time prolongation, however, could lead to false positive weak reactions, as observed in some of our M SSA isolates (Table 2). Thus, as shown in our study and recommended by others only test cards showing a strong agglutination pattern within 3 minutes should be considered positive.\(^{15}\)

In conclusion, the M RSA-Screen latex kit is an easily performed assay that is suited for clinical microbiology laboratories that provide rapid and reliable detection of M RSA, valuable information for proper treatment, and specific infection control measures.

Part of this work was presented at the ASM 102nd General Meeting, Salt Lake City, Utah May 19-23, 2002. This study was supported by a research grant from the AUB Chairman’s fund.

REFERENCES


<table>
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<tr>
<th>Table 3. Percent sensitivity, specificity, and predictive values positive (PPV) and negative (NPV) for the M RSA-Latex</th>
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<tr>
<td><strong>Aspect</strong></td>
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<tr>
<td>Sensitivity</td>
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<tr>
<td>Specificity</td>
</tr>
<tr>
<td>PPV</td>
</tr>
<tr>
<td>NPV</td>
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* Calculations were done based on the findings in Table 1 according to reference number 9.
Hepatitis C virus (HCV) is a blood-borne virus that infects the liver. HCV affects millions of Americans, and poses a serious public health threat with sequelae such as cirrhosis, hepatocellular carcinoma, and liver failure. This paper reviews means of transmission, characteristics of the various risk groups, and clinical presentations of both the acute and chronic stages of HCV infection. Diagnostic methods, including screening and confirmatory tests, along with relevant clinical and physiologic findings are also described. Additionally, treatment strategies, in particular combination therapy with interferon-α-2b and ribavirin, are discussed. Contraindications, side effects, and monitoring of this therapeutic modality are considered. Finally, prospective treatments are presented.

**ABBREVIATIONS:** ALT = alanine aminotransferase; AST = aspartate aminotransferase; EIA = enzyme immunoassay; FDA = Food and Drug Administration; GGT = gamma glutamyltransferase; HCV = hepatitis C virus; NAT = nucleic acid amplification testing; PKR = RNA-dependent protein kinase; PT = prothrombin time; RIBA = recombinant immunoblot assay; RT-PCR = reverse transcription-polymerase chain reaction; URL = upper reference level.

**INDEX TERMS:** hepatitis C virus; hepatocellular carcinoma; interferon; peginterferon; ribavirin.

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Hepatitis C is the most common blood-borne infection and is the leading cause of chronic liver disease. In the United States, approximately four million people are infected with Hepatitis C virus. Nearly three million of those are in the chronic stage and about forty thousand new cases occur each year. HCV infection has a 1% to 2% mortality rate with approximately 10,000 deaths occurring annually in the United States. One of several hepatitis viruses (A through G), hepatitis C accounts for 20% of viral hepatitis cases and is potentially the most harmful to the host because of its propensity to cause chronic infection and severe liver damage often requiring liver transplants.

The hepatitis C virus (HCV) is a small, enveloped, single-stranded RNA virus of the family Flaviviridae, and has a genome of 9.6 kilobases coding for structural and non-structural proteins. HCV has a high rate of mutation and genomic heterogeneity (the greatest variability is seen in the envelope glycoproteins).

Genomic sequencing by reverse transcription-polymerase chain reaction (RT-PCR) has lead to the identification of several HCV genotypes. The genotypes vary in geographic distribution, viral RNA levels, clinical severity, and response to treatment. Simmonds nomenclature describes six genotypes, some of which have closely related subtypes, e.g., 1a-c, 2a-c, 3a-b, 4a, 5a, and 6a. Other sources report that 11 genotypes and greater than 90 subtypes exist. Genotypes 1a (58%), 1b (22%), 2b, and 3a are the most common genotypes in the United States and are most commonly associated with chronic hepatitis. Genotype 1b produces the most severe presentations such as hepatocellular carcinoma and cirrhosis and is least responsive to therapy, i.e., 40% response rate. Genotypes 2b and 3 are most responsive to therapy, i.e., 80% response rate, and successful treatment can occur within 24 weeks. The highest viral RNA levels are associated with 1b, while the lowest involve 2b and 3a.
Because of the ability of the virus to alter its viral envelope proteins, the rapid, spontaneous rate of mutation, and the inability of the host’s immune system to target the virus effectively, i.e., protective antibodies able to neutralize the virus are not produced by the host, the virus efficiently can infect and re-infect the host multiple times. Moreover, the mutability of HCV proteins make production of a vaccine extremely problematic; hence, no vaccine is currently available.

TRANSMISSION
HCV is transmitted by parenteral exposure to infected blood. Routes of infection include organ transplantation, IV drug use, sexual contact, exposure to blood, perinatal transmission, or occupational exposure. Blood transfusion is another source, and in the U.S. 3% of donors are positive for HCV antibodies. However, because of improved screening methods initiated in 1992, the incidence of infection due to blood or blood product transfusion or organ transplantation has greatly decreased.

The highest risk group for HCV are intravenous drug users (60% of cases). Other high risk groups are recipients of clotting factors made before 1987, and those who received blood transfusions prior to 1992. Healthcare workers and sexual contacts of infected persons are considered to be at a low, but not insignificant, risk for contracting the virus. In 10% of cases, the source of infection is unidentified, but these cases are thought to occur by exposure to the virus through cuts or wounds, and the fact that the virus can remain viable for weeks outside of a host may be relevant in these cases.

CLINICAL PRESENTATIONS
The incubation period for HCV ranges from 40 to 120 days. Two types of HCV infection occur, acute and chronic. The clinical presentations of both types of HCV infections vary. The acute stage may be asymptomatic, or may involve jaundice, anorexia, malaise, abdominal pain, fatigue, migraines, nausea, or dark urine. Liver enzyme levels in the serum may be elevated, and damage to liver cells may be detectable at fifty days. In 15% of acute cases, the infection resolves within five months and liver enzymes return to normal levels suggesting full recovery. Full recovery is defined by absence of HCV RNA in serum and normal levels of the liver enzyme, alanine aminotransferase (ALT). Unfortunately, 85% of acute infections result in chronic disease and are signaled by persistent elevation or fluctuating levels of ALT for greater than six months. A very small percentage of acute cases culminate with fatal liver destruction.

Chronic HCV infection is typically insidious, progressing at a slow rate without symptoms in most patients during the first two or more decades after initial infection. Symptoms ultimately result from the cytopathic effect of the virus on the liver over time by a constant, low rate of hepatocyte destruction. An inflammatory reaction to the infection within the liver is also evident. Symptoms can vary. Patients may be asymptomatic for years, frequently 10 to 20 years, and then enter a chronic stage. Those persons with chronic infection are at risk for one of the following: fibrosis, cirrhosis, hepatocellular carcinoma, or liver failure. In addition, patients may present with hepatosplenomegaly, muscle wasting, portal hypertension, or ascites. Subsequent systemic abnormalities may develop that include cutaneous, endocrine, hematological, renal, and circulatory abnormalities.

Serum liver enzyme levels are typically increased during this chronic stage. An elevation of these enzymes for more than six months, coupled with detection of antibodies to HCV, i.e., anti-HCV, is characteristic of chronic hepatitis. Factors associated with more severe liver injury due to HCV include advanced age, alcoholism, and immunosuppression.

DIAGNOSIS
Diagnosis of HCV infection relies on detection of antibodies to HCV via screening and confirmatory tests, i.e., detection of HCV RNA in serum by RT-PCR or use of a recombinant immunoblot assay. Clinical presentation and risk factors, e.g., exposure, are also taken into consideration when making a diagnosis. Indirect evidence of liver damage can be assessed by chemistry profiles that measure liver enzymes; while direct evidence of liver damage can be ascertained by liver biopsy.

Enzyme immunoassay (EIA) serves as the screening test and detects antibodies to HCV. An EIA acute panel for viral hepatitis includes tests for hepatitis A, hepatitis B, and hepatitis C. Individuals with HCV will test positive for anti-HCV, which is detectable in 80% of patients by six weeks, and in 90% of patients by twelve weeks. Presence of anti-HCV does not confer long-term immunity to HCV, and persistence of this antibody indicates that actively replicating HCV remains in the patient’s body.

The FDA approved an EIA test for antibodies to HCV in May 1990, followed by a more sensitive and reliable test introduced in July 1992. In 1996 a modified second-generation test (anti-HCV 3.0) was introduced to screen all blood supplies for antibodies to HCV. However, these very sensitive tests suffer from false-positive results and require supplemental confirmatory tests to eliminate them. In 1999, FDA approved an improved test for confirming positive results from
screening tests. In the same year, blood transfusion centers began nucleic acid amplification testing (NAT) of pooled donor samples for human immunodeficiency virus (HIV) and HCV under FDA's Investigational New Drug application. In 2002, the FDA approved the NAT test, Procleix HIV-1/HCV assay, which directly detects the genetic material of all HCV and HIV genotypes in pooled donor samples. If viral nucleic acid is detected using NAT, then the blood donor units making up the pool are retested individually to determine which unit contains the virus. Once determined that an individual unit is contaminated by HIV or HCV, it is taken out of the blood supply; thus stopping transmission of the virus to blood recipients. NAT is more effective than antibody testing since it decreases the window period for detection of HCV infection, i.e., from 70 to 80 days time between exposure to the virus and when antibodies to the virus become detectable, to 30 days. The Procleix HIV/HCV assay, developed by Gen-Probe Inc., is now being manufactured and marketed by Chiron Corporation worldwide. Presently 75% of the nation's blood supply is being screened by the Procleix™ system. This system is restricted to screening blood donors and is not being used to diagnose individuals with HCV.

Results from individuals who test positive for HCV antibodies using EIA screening techniques are verified using methodologies with increased specificity. For confirmatory testing, a recombinant immunoblot assay (RIBA) is most often used. RIBA is a type of Western blotting, and like the EIA, it tests for the presence of antibodies against HCV. In patients not producing significant amounts of antibodies, RT-PCR may be used as the confirmatory test because of its increased sensitivity. RT-PCR directly measures HCV virions in serum by detection of HCV RNA genome. The test measures viral load which is helpful in predicting response to treatment. RT-PCR for HCV may be positive two weeks following the initial infection. A qualitative RT-PCR assay for HCV, the COBAS Amplicor® HCV test manufactured by Roche Molecular Systems, Inc, has recently been approved by the FDA. A positive test indicates active infection, i.e., replicating virions in the serum.

Hepatitis C infection may produce characteristic serum chemistry findings. These findings can aid in diagnosis and provide, along with liver biopsy, an estimation of the extent of liver damage. Table 1 shows serum chemistry findings that occur with HCV infection. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are markedly increased, i.e., 0 to 20 times the upper reference level (URL). In acute hepatitis, ALT is frequently increased greater than 10-fold URL, while AST is increased more than 3-fold URL. In chronic hepatitis increases are generally more modest. Alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) levels may also be increased but usually less than 2-fold URL. Increases in ALP and GGT may indicate that liver damage has occurred, e.g., cirrhosis. Also reflecting liver damage, total serum bilirubin levels are typically increased, while direct (conjugated) and indirect (unconjugated) bilirubin levels are either increased or normal. Additionally, serum ammonia levels are increased, serum urea levels are decreased, and the blood urea nitrogen/creatinine ratio will be low. Albumin and total protein levels may be normal in acute hepatitis, but are often decreased in chronic hepatitis.

Other changes are seen in urine and hematological parameters. Urinalysis is typically positive for bilirubin and normal or increased for urobilinogen. The amount of bilirubin...
PEGINTERFERON ALFA-2B has been approved for use in chronic hepatitis C in the United States. Formerly monotherapy typically lasted six months to two years, with a low success rate of less than 15%. However, an improved success rate is seen with a branched-chain, polyethylene glycol modified form of interferon, peginterferon α administered for 48 weeks. Because of relatively low success rates of monotherapy with different interferon formulations, combination therapy with additional therapeutic agents is recommended, unless contraindications to those supplemental agents exist (contraindications will be discussed later).

Combination therapy consists of interferon α injections and oral ribavirin. Prior to June 1998, the FDA approved the combination treatment as a secondary treatment regimen only when interferon alone failed. However, in June 1998 the FDA approved combination therapy as a primary course of treatment and as of September 2002 the optimal regimen is a 24- to 48-week course of combination peginterferon alfa-2b (1.5 mcg/kg/week) and ribavirin (400 mg twice/day). Safe dosage levels of ribavirin have not yet been determined for pediatric patients; in these cases monotherapy is indicated. Ribavirin is a synthetically produced nucleoside that inhibits a broad spectrum of viruses by inhibiting viral DNA and RNA synthesis and replication. Overall, combination therapy, as compared with monotherapy, has both a higher rate of elimination of HCV RNA (up to 70% of patients), and a lower rate of relapse following completion of therapy.

Among patients who become HCV RNA negative during treatment, a proportion will relapse when therapy is stopped. Undetectable levels of HCV RNA for at least six months following completion of therapy indicate sustained response to therapy. Recommended duration of therapy is six months up to a year, depending on HCV genotype. This regimen has been shown to be effective in approximately 40% of patients, resulting in the elimination of the virus and improvement in liver inflammation (indicated by a decrease in liver enzyme levels in the serum). HCV genotypes 2 and 3 are most responsive to therapy, and a 24-week course of combination therapy (70% to 80% response rate) yields similar results to those of a 48-week course. On the other hand, HCV genotype 1 is less responsive (40% to 45% response rate) to combination therapy and a 48-week course is necessary for a more sustained response rate.

Contraindications for use of interferon α-2b and peginterferon include conditions such as cirrhosis, ascites, persistent jaundice, and wasting; age greater than sixty years; or concurrent HIV infection with low CD4 levels. Additional contraindications include evidence of neuropsychological problems, recent alco-
hol abuse (must abstain for at least six months prior to treat-
meth), autoimmune disease, and bone marrow hypoplasia.
Contraindications for use of ribavirin include anemia, renal
dysfunction, coronary artery disease, or cerebrovascular disease.

Side effects of interferon use include fatigue, flu-like symp-
toms, depression and other psychiatric disorders, rashes, birth
defects, exacerbation of autoimmune disease or existing heart
conditions, and suppression of bone marrow hematopoe-
sis. Bone marrow suppressive effects appear to be more com-
mon with peginterferon than standard interferon; thus, fre-
quent monitoring of cell counts is recommended for patients
receiving this treatment. These adverse effects occur in more
than 10% of patients and vary from mild to severe, but can
be mitigated by adjusting the dosage of interferon adminis-
ter. Most side effects are worse during the first few weeks
of treatment and may diminish as treatment is continued.

Undesirable reactions related to ribavirin include anemia
(causes RBC hemolysis), ischemia, fatigue, itching, i.e., his-
tamine-like effect, and rashes. These effects, too, can some-
times be relieved by dosage adjustment. Ribavirin is excreted
mostly by the kidneys and patients with serum creatinine
levels above 2.0 mg/dL should not given this drug. Ribavirin
is contraindicated in pregnancy. Less common side effects
of ribavirin include severe bacterial infections, marked throm-
bocytopenia or neutropenia, seizures, and retinopathy. Ad-
verse effects of ribavirin are most severe early in treatment,
and may be more severe when combined with interferon.
Fatal myocardial infarctions and strokes have been reported
with combination therapy.

During treatment, the patient should be closely monitored
at regular intervals to assess side effects and response to treat-
ment. Blood counts and aminotransferase (AST and ALT)
levels should be measured at weeks 1, 2, 4, and at 4- to
8-week intervals subsequently. If side effects are manifest, drug
dosages should be adjusted to alleviate them. A serious and
rare side effect known as autoimmune hepatitis can be in-
duced by treatment. This can be avoided by monitoring ALT
levels and if they rise to greater than twice the baseline value,
therapy should be stopped and the patient monitored. Corti-
costeroid therapy may be needed to control the hepatitis.

Because disease progression and response to therapy is affected
by HCV genotype, initial evaluation for genotype is recom-
mended. HCV RNA should be measured at 24 weeks. If at
this time HCV RNA is still detected, therapy should be
stopped and considered unsuccessful. If HCV RNA is not
detected at 24 weeks, and genotypes 2 and 3 are implicated,
then treatment is successful. If genotype 1 is implicated and

**Algorithm for HCV treatment**

1. Make diagnosis based on AST and ALT elevations,
   anti-HCV, HCV RNA in serum, and chronic
   hepatitis shown by liver biopsy.
2. Assess patient for suitability of therapy and
   contraindications.
3. Test for HCV genotype.
4. Discuss side effects and possible outcomes
   of treatment.
5. Start therapy with peginterferon α-2b dose of 1.5
   mcg/kg weekly and oral ribavirin 800 mg daily.
6. At weeks 1, 2, and 4 and then at intervals of every 4
   to 8 weeks thereafter, assess side effects, symptoms,
   blood counts, AST, and ALT levels.
7. At 24 weeks, assess AST and ALT levels and HCV
   RNA. In patients with genotypes 2 and 3, stop
   therapy. In patients with genotype 1, stop therapy if
   HCV RNA is still positive, but continue therapy for
   a total of 48 weeks if HCV RNA is negative, retest
   for HCV RNA at the end of treatment.
8. After therapy, assess AST and ALT levels every two
   months for six months. In responders, repeat HCV
   RNA testing six months after stopping therapy.

Adapted from: http://www.niddk.nih.gov/health/digest/pubs/
HCV RNA is not detected, therapy should be continued for an additional 24 weeks to prevent recurrence.

Long term monitoring is recommended. Following successful treatment, AST and ALT levels should be monitored every two months up to six months. Six months after stopping therapy, testing for HCV RNA by RT-PCR should be performed. If HCV RNA is still negative, relapse is unlikely. Figure 1 summarizes the treatment protocol.

Few approved options exist for those patients who fail treatment or relapse. As mentioned, peginterferon α may be more successful, and has fewer side effects than interferon α-2b. Currently, the FDA is considering approval of peginterferon combined with ribavirin. Treatments on the horizon include recombinant interleukin 10 (and other cytokines), and antiviral agents that inhibit HCV-derived enzymes, such as RNA polymerases, helicases, and proteases. Also being investigated are drugs including ribozymes (enzymes designed to breakdown viral RNA) and antisense oligonucleotides.

Specific mechanisms being researched and targeted for antiviral action are blockage of HCV antigen production, inhibition of viral glycosylation, and blockage of viral entry into cells. As a last resort, liver transplantation may be the only effective treatment for HCV. However, the new liver frequently becomes infected. Liver transplantation is still considered beneficial because the patient may live the remainder of his/her life without complications due to the insidious course of the disease.

**SUMMARY**

Although information continues to accumulate about HCV and treatment strategies continue to improve, hepatitis C remains a serious health threat. Current combination therapy has a reasonably good response rate and represents an improvement over prior strategies; however, treatment failures and serious side effects do occur, highlighting the need to research and design newer treatments. As a result of the successful cloning of the hepatitis C virus in 1991, researchers have been striving to develop a vaccine, but their efforts are complicated by the need to include all HCV genotypes to make an effective vaccine. Because HCV mutates rapidly within infected patients, an effective vaccine may become useless if new strains of mutant viruses arise. Thus, alternative strategies are needed to meet the challenge of HCV infection.

**ACKNOWLEDGMENTS**

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**REFERENCES**

New reports of serious complications from Group C (GCS) and Group G streptococci (GGS) with probable respiratory entry have been described. Particularly interesting are cases of toxic shock-like syndrome and rheumatic fever in previously healthy patients. Serious GCS and GGS infections will be missed where selective methods for group A streptococci (GAS) only are used on throat specimens.

**ABBREVIATIONS:** ASO = anti-streptolysin O; BAP = blood agar plate; GAS = group A streptococci; GCS = group C streptococci; GGS = group G streptococci; OIA = optical immunoassay.

**INDEX TERMS:** streptococcus infections.

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Group A streptococci (GAS) are considered the primary pathogenic β-hemolytic streptococci cultured from the throat. GAS throat infections are routinely treated to reduce spread to patient contacts, and to reduce supplicative and non-suppurative complications. Standard protocol is to screen with a rapid test for GAS and perform throat cultures on specimens with negative screening results.

Although GAS are the most frequent streptococci isolated from throat cultures, β-hemolytic GCS and GGS and hemolysin deficient variants cause epidemics of exudative pharyngitis/pharyngotonsillitis. The rate of positive throat cultures that are GCS rather than GAS positive in certain seasons and geographical areas ranges from 1% to 11%. In addition, GCS and GGS are implicated as a cause of serious complications including rheumatic fever in previously healthy children and adults. Notably, pharyngeal carriage of GGS and/or GCS and not GAS preceded several rheumatic fever cases.

The main focus of this paper is the serious complications and sequelae from GCS and GGS in previously healthy adults with confirmed or probable respiratory entry. A patient's quality of life may be permanently impaired because of clinical practices that ignore GCS and GGS unless the specimen comes from a normally sterile site.

**LABORATORY DIAGNOSIS OF GAS, GCS, AND GGS**

Standard practice for patients with pharyngitis or pharyngotonsillitis is to obtain a throat swab, then either culture directly or screen for GAS by a rapid test method and perform throat cultures on the negative GAS screens.

Throat specimens are cultured on trypticase soy agar with 5% sheep blood (BAP) at 35 °C overnight in aerobic conditions, and β-hemolytic colonies are subcultured onto fresh 5% BAP with a 0.04U Bacitracin disk. Bacitracin sensitive streptococci in throat specimens are reported as presumptively GAS because 95% of GAS are sensitive and only 9% of non-GAS are sensitive. Bacitracin sensitivity and β-hemolysis are seen with up to 6% of the GCS isolates and 8% to over 50% of specific GGS isolates. Whether Bacitracin sensitive or resistant, GCS and GGS are potential pathogens and yet, are not routinely identified from throat specimens.
The diagnosis of streptococcal throat infections is based on a wide variety of approaches throughout the U.S. Some pediatricians only screen for GAS with rapid tests, some only do cultures, others screen and backup with culture, a few do both, and a few neither. Illustrating differences within one small region, survey results from six microbiology laboratories in Northern Illinois showed that in 2001, most laboratories do direct antigen testing for only GAS. Although not routinely performed anymore, when throat specimens are streaked onto BAP, most of these laboratories are reporting non-GAS if they are present in significant numbers. These non-GAS infections are not usually speciated or grouped. Only two laboratories reported further grouping non-GAS.

When more serious complications occur, testing includes a wide variety of protocols. For specimens from sterile sites, preliminary serological or biochemical testing for β-hemolytic streptococci includes grouping the C antigen on the surface of the organism or biochemical testing. Agglutination tests may be performed to identify the Lancefield group as one of A-G. Biochemical testing to confirm group A includes bacitracin sensitivity, sulfamethoxazole-trimethoprim (SXT), or pyrrolidonyl-β-naphthylamide (PYR) hydrolysis. PYR has been shown to be the most specific and sensitive if used alone. Table 1 contains biochemical reactions used to differentiate group A, C, and G β-hemolytic streptococci.

β-hemolytic GCS streptococci include S. equi, S. equisimilis, and S. zooepidemicus. With the exception of S. zooepidemicus, GCS strains produce hemolysins that cross-react with streptolysins produced by GAS. Patients infected with S. equi or S. equisimilis often have significant ASO titers, and both organisms can be bacitracin sensitive. Lancefield grouping is the best method to definitely identify the organism as group A, C, or G.

### Table 1. Biochemical reactions of β-hemolytic group A, C, and G streptococci

<table>
<thead>
<tr>
<th>Organism</th>
<th>Baci*</th>
<th>SXT†</th>
<th>PYR‡</th>
<th>Sorb§</th>
<th>Treh§</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes (gr A)</td>
<td>S (99%)</td>
<td>R</td>
<td>P</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>S. anginosus (gr G)</td>
<td>R (99%)</td>
<td>S</td>
<td>N</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>S. equi (gr C)</td>
<td>S (80%)</td>
<td>S</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>S. equisimilis (gr C)</td>
<td>R (99%)</td>
<td>S</td>
<td>N</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>S. zooepidemicus (gr C)</td>
<td>S (71%)</td>
<td>S</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
</tbody>
</table>

* Baci = bacitracin
† SXT = sulfamethoxazole-trimethoprim
‡ PYR = pyrrolidonyl-β-naphthylamide
§ Sorb = sorbitol
§+Treh = trehalose

Serious complications of GCS and GGS infections

Cases of exudative pharyngitis from GCS and GGS are now being recognized in specific regions of the U.S. and in other countries. Sequelae and serious complications once believed to be exclusively from GAS are now confirmed to be from GCS or GGS streptococci. GCS and GGS are now being recognized in specific regions of the U.S. and in other countries. Sequelae and serious complications once believed to be exclusively from GAS are now confirmed to be from GCS or GGS streptococci. The M protein of GGS or GCS matches the M protein of GGS or GCS streptococci are β-hemolytic, bacitracin resistant (like most group B), or sensitive (like group A), and sodium hippurate positive (like group B). Thus, they may resemble group B or group A, depending upon the biochemical tests used. In addition, the M protein of GGS or GCS matches those of particular strains of group A streptococci that cause rheumatic fever. The M protein is a part of the pill, which is a potent virulence factor that resists phagocytosis. GGS also have a cross-reactive streptolysin O, so ASO and DNase B titers can rise as a result of GGS infections.

### DISCUSSION

Serious complications of GCS and GGS infections

Cases of exudative pharyngitis from GCS and GGS are now being recognized in specific regions of the U.S. and in other countries. Sequelae and serious complications once believed to be exclusively from GAS are now confirmed to be from GCS or GGS streptococci. Three of these patients developed post-streptococcal glomerulonephritis. Anti-streptolysin O (ASO) titers were done on serum from the family and the results were nega-
tive. (S. zooepidemicus produces a soluble hemolysin, which does NOT cross-react with the hemolysins of GAS.) In another outbreak of pharyngitis (85 patients), S. zooepidemicus infection resulted from the ingestion of raw dairy products. Thirty-three percent of these patients developed glomerulonephritis. Certain strains of S. zooepidemicus can produce endostreptosin (ESS), a cytoplasmic polypeptide associated with nephritogenic strains of group A streptococci.37

Serious infections and complications from GCS and GGS have been reviewed in papers published from 1979 to present.22,36-41 GCS and GGS with nephritogenic or rheumatogenic M types homologous with GAS have been identified.22,42-44 GCS and GGS are described in reviews as also the cause of serious infections in compromised patients including toxic shock-like syndrome, necrotizing fasciitis, exophthalmitis, puerperal fever, pharyngitis, epiglottitis, pneumonia, impetigo, cellulitis, empyema, septicemia, endocarditis, septic arthritis, osteomyelitis, abscesses, and meningitis (Table 2).36,38-41 Cases of young healthy adults and children with life threatening toxic shock-like syndrome, meningitis, and septicemia or sequelae from GCS or GGS are changing the previous paradigm.17-28 These organisms can no longer be considered benign for healthy individuals.

**Therapy**

Rapid diagnosis of GAS infections is important because of the serious sequelae, including rheumatic fever and glomerulonephritis that may develop. Rheumatic fever is preventable by early treatment of the initial pharyngeal infection. Although glomerulonephritis is not preventable, early intervention may prevent kidney failure.45 Rapid diagnosis of GCS and GGS infections that lead to glomerulonephritis, toxic shock-like syndrome, and rheumatic fever may also prevent unnecessary death and disability. Treatment depends upon the nature and severity of the GCS or GGS infection.

GCS and GGS do not respond as readily to penicillin G as GAS and are more likely to be resistant to penicillin and vary with resistance to erythromycin and other antimicrobials.47-53 GCS and GGS may be bacitracin sensitive or resistant and both appear to be sensitive to penicillin in vitro; however, in vivo the response is often delayed, and other drugs (especially aminoglycosides) are recommended to effect a cure.53 Penicillin tolerance by GCS and GGS is on the rise. Current literature recommends a combination of penicillin G and gentamicin (aminoglycoside) or other antimicrobials for serious GCS streptococcus infections.47-53

This in vivo delayed therapeutic response to penicillin in GCS and GGS is in direct contrast to the quite likely immediate in vivo response of GAS to this drug. Therefore, Lancefield grouping of clinical isolates could potentially decrease morbidity in those patients with infections due to GCS or GGS who require more than penicillin in order to recover. GCS infections appear to follow certain patterns. In the past, the most common outcome of GCS infections treated with penicillin G is that the patient recovers from the initial pharyngitis or pyoderma. About a third of patients with pharyngitis infections by nephritogenic strains develop glomerulonephritis.37,43 Septicemia, endocarditis, toxic shock-like syndrome, and meningitis are all serious complications of GCS and GGS infections that are frequently fatal. With the increase in penicillin tolerance or resistance and erythromycin resistance in GCS and GGS infections, an increase in serious complications is likely. Pharyngitis will be treated only if it is due to GAS by current protocols of diagnosing pharyngitis. The most frequently prescribed drug for GAS is penicillin. Therefore, a change of practice to rapid identification of the GCS or GGS and the initiation of dual therapy despite extreme in vitro sensitivity to penicillin alone, should improve the recovery chances of these patients.53

**CONCLUSIONS**

GAS, GCS, and GGS have been reported as transient inhabitants of healthy throats.12 Evidence of throat carriage of rheumatogenic strains of GCS and GGS may indicate that carriage is not benign. Reports of healthy children and adults

<table>
<thead>
<tr>
<th>Table 2. Infections, complications, and sequelae caused by GCS or GGS</th>
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<tr>
<td>Initial infection with potential sequelae</td>
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<tr>
<td>Pharyngitis</td>
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<tr>
<td>Sequelae</td>
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<tr>
<td>Glomerulonephritis</td>
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<tr>
<td>Rheumatic fever</td>
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<tr>
<td>Other infections and complications</td>
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<tr>
<td>Abscesses</td>
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<tr>
<td>Cellulitis</td>
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<tr>
<td>Empyema</td>
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<tr>
<td>Endocarditis</td>
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<tr>
<td>Epiglottitis</td>
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<tr>
<td>Exophthalmitis</td>
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<tr>
<td>Impetigo</td>
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<tr>
<td>Meningitis</td>
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<tr>
<td>Necrotizing fasciitis</td>
</tr>
<tr>
<td>Nephritic syndrome</td>
</tr>
<tr>
<td>Osteomyelitis</td>
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<tr>
<td>Otitis media</td>
</tr>
<tr>
<td>Periperal fever</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Septic arthritis</td>
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<tr>
<td>Septicemia</td>
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<tr>
<td>Toxic shock-like syndrome</td>
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contracting life-threatening GCS or GGS infections with oral or respiratory entry (confirmed or suspected) suggests that throat specimen screening for GAS may be insufficient for certain populations.

In a recent case, throat cultures on a previously healthy woman were reported as having β-hemolytic colonies that were not group A streptococci. She was not treated. The patient developed bacteraemia and meningitis a few days later and S. zooepidemicus was isolated from her cerebrospinal fluid. She was then asked if she had unusual pets and reported having goats. Her neural function appears to be permanently impaired. Perhaps, she would be healthy if her throat culture results had been taken seriously.

Replacement of throat cultures with GAS antigen screening or DNA hybridization tests should be reconsidered especially in clinics serving rural communities or areas known to have higher rates of GGS or GCS throat infections. Instead, throat cultures from farmers, veterinarians, rural patients, and known endemic populations should be actively tested for GCS and GGS by Lancefield agglutination tests or other antigen specific tests when suspicious colonies appear on BAPs. To assure that each patient is provided the best chance of correct diagnosis and therapy, the following protocol is recommended:

1. screen for GAS with highly sensitive and specific rapid test methods;
2. culture negative screens and collect data for a region to determine rate of positives for GAS, GCS and GGS;
3. if throat infections and serious complications, presumably from GAS, GCS and GGS are missed by the rapid antigen tests, continue BAP cultures of throat specimens;
4. treat GCS and GGS as potentially as pathogenic GAS;
5. in addition to asking about exposures to humans with infections, also ask patients for information on exposure to zoo or farm animals or unpasteurized milk products;
6. speciate the infectious organism when the answer is yes;
7. do antimicrobial susceptibility testing for GCS or GGS infections and treat more aggressively for GGS or GCS especially when S. zooepidemicus found.

When infections are positive for GCS or GGS, antibiotics will help prevent life threatening complications. For specific populations, replacing standard throat cultures as a method of identifying throat pathogens with antigen based tests or selective agar cultures that only identify GAS, leaves patients at risk of being misdiagnosed or undiagnosed resulting in treatment delays.

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Obtaining a Clinical Laboratory Science Degree via Distance Technology

SUZANNE CAMPBELL

OBJECTIVE: To identify institutions and program officials associated with clinical laboratory science (CLS) academic programs available via distance technology; to collect and summarize data from these programs with regard to on-line instructional methodologies; to determine the level of success of educational strategies and methodologies utilized in on-line CLS programs; to determine the feasibility of developing an on-line program at Seward County Community College (SCCC), Liberal, Kansas.

DESIGN: An on-line CLS program survey tool was sent to eight higher education institutions which had previously indicated that they offer a CLS academic program at the associate, bachelor, or master level via distance technology. Program officials were asked to answer questions pertaining to areas such as program format, on-line admission requirements, program costs, student costs, faculty workload, and on-campus versus on-line student performance.

SETTING AND PARTICIPANTS: The survey was sent to eight program officials who identified their institutions as having a CLS program available through distance technology.

MAIN OUTCOME MEASURES: Responses from current distance technology CLS program officials were collected and tallied. Responses were recorded as ‘yes’ or ‘no’ in categories such as program format, program and student costs, and comparison of on-campus versus on-line student performance. The two groups of students were compared in areas of success rate, retention rate, graduation rate, external certification pass rate, employment placement rate, and employer satisfaction level.

RESULTS: The response rate for the survey was 87.5% (7/8). Program officials indicated that various educational methodologies were incorporated in providing CLS education via distance technology. All of the respondents utilize some type of Web-enhanced, Internet-based access to deliver course material. Clinical laboratory procedures are taught via instruction within a cooperative laboratory, program clinical affiliate laboratory, or during on-campus student laboratories. Program officials indicated that student enrollment has increased due to the availability of the distance technology. Students enrolled via distance technology perform as well or better than the on-campus students on certification exams and in the clinical setting. Data from these institutions indicate that it is feasible to develop an on-line program at SCCC in an effort to increase student enrollment.

CONCLUSION: The results indicate that CLS programs which offer the curriculum via distance technology have experienced increased student enrollment thus graduating more students to fill the employment needs. These current distance technology programs are leading the future trends in CLS education of the 21st century.

ABBREVIATIONS: AD = associate degree; BS = Bachelor of Science degree; CLS = clinical laboratory science; CLS/MT = clinical laboratory scientist/medical technologist; CLT/MLT = clinical laboratory technician/medical laboratory technician; MS = Master of Science degree; SCCC = Seward County Community College.

INDEX TERMS: articulation; CLS degree; distance technology; Web-enhanced programs.

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The purpose of this research project was to gather comprehensive data pertaining to the implementation and success of Web-enhanced CLS programs at the Bachelor of Science (BS) - clinical laboratory scientist/medical technologist (CLS/MT) level and the associate of science (AS) - clinical laboratory technician/medical laboratory technician (CLT/MLT) level. This data will be utilized to develop and implement a Web-enhanced option for the CLT/MLT program at Seward County Community College (SCCC), Liberal KS. The CLT/MLT program at SCCC is a well-established pro-
program with a strong academic course of study and a reputation of graduating competent entry-level CLTs/MLTs. Currently the CLT/MLT program at SCC C has a low number of students enrolled in the program. In order to make the program fiscally responsible, the number of students enrolled in the program needs to increase. Upon the review of options to increase enrollment, it was determined by input from the program advisory board and faculty, that offering the CLT/MLT courses via a Web-enhanced method is the best course of action. The Web-enhanced option will allow the CLT/MLT program to serve new students at a distance and to better serve the existing populations.

EMPLOYMENT OPPORTUNITIES

In June 2001, the American Hospital Association (AHA) reported a 12% vacancy rate in the laboratory workforce based on the percent of unfilled, budgeted positions. By comparison, there was an 11% vacancy rate for registered nurses. This survey reported that the shortage of clinical laboratory professionals is getting worse and affecting access to care in hospitals across America. Other surveys have reported a laboratory personnel shortage as high as 20%. The AHA also reported that 43% of all hospitals were having more difficulty than last year recruiting selected healthcare professionals which include laboratory personnel. The Bureau of Labor Statistics has projected a 17% growth rate in the number of laboratory personnel between 1998-2008. This is compared to a growth rate of 14.4% for other professions. During this period, there will be 53,000 new jobs in the field with 40,000 vacancies due to retirement and employees leaving the field. This equals 93,000 total positions, thus the need for 9,300 positions per year. Currently there are only 4,110 total graduates per year from CLS/MT and CLT/MLT programs. This leaves 5,190 unfilled positions per year in the field of medical technology. The current vacancy rate for CLTs/MLTs in Oklahoma is 14.3% and in Kansas it is 15.8%.

CLTs/MLTs are trained to perform a variety of laboratory tests. These tests include the typing and crossmatching of blood and blood components; drug monitoring (both for therapeutic monitoring of drugs and detection of drugs of abuse); identifying anemias, leukemias, and other blood disorders; identifying infectious organisms and determining their susceptibility to specific antibiotics; and performing procedures for various blood and body fluid analysis. In this time of concern regarding bioterrorism, CLSs/MTs and CLTs/MLTs are the laboratory personnel who will be isolating and identifying the anthrax bacillus and other biological weapons. It is estimated that 80% of all physician decisions are based on laboratory test results produced by CLSs/MTs and CLTs/MLTs. Without these results, the physician would be unable to accurately diagnose, and in many cases, effectively treat the patient.

ON-LINE LEARNING

According to William A Draves, the author of Teaching Online, half of all learning in the 21st century will be on-line. On-line learning will be better than in-person learning for obtaining cognitive skills. On-line learning allows the student to determine their peak learning time and to learn at their own speed. On-line learning allows more interaction; thus 100% of students can be talking on-line versus the 33% that will participate in a traditional face-to-face course. Course participants will be worldwide with the instructors being the foremost experts and authorities in their respective fields. On-line courses will become less expensive and will offer more resources and ways to learn on-line.

For students to be successful in a Web-enhanced course, they must be result oriented, self motivated, and demonstrate self-discipline. They must have time management skills which allow them to stay on task without direct supervision. They must have a manageable balance of academic course work, employment, and family commitments.

The student must also have access to a computer with reliable Internet service. The student must possess basic computer skills to install software; create, save, and manage files; post text to an asynchronous threaded discussion; and send and receive e-mail.

BEST PRACTICES

The Higher Learning Commission has published a document titled Best Practices for Electronically Offered Degree and Certificate Programs. These Best Practices were developed by the eight regional accrediting commissions in response to higher education institutions offering technology-mediated instruction at a distance. The Best Practices are intended to assist higher education in planning distance education activities for electronically offered programs and to provide an assessment framework once the program is implemented. The Best Practices are identified as:

- education is best experienced within a community of learning where competent professionals are actively and cooperatively involved with creating, providing, and improving the instructional program;
- learning is dynamic and interactive, regardless of the setting in which it occurs;
• instructional programs leading to degrees having integrity are organized around substantive and coherent curricula which define expected learning outcomes;
• institutions accept the obligation to address student needs related to, and to provide the resources necessary for, their academic success;
• institutions are responsible for the education provided in their name;
• institutions undertake the assessment and improvement of their quality, giving particular emphasis to student learning; and
• institutions voluntarily subject themselves to peer review.8

The Best Practices can be divided into five separate components: institutional context and commitment, curriculum and instruction, faculty support, student support, and evaluation and assessment. Through the implementation of a Web-enhanced option for the CLT/MLT program at SCCC, the college is further supporting its mission statement with regard to providing higher education as an investment in future societies and as a college that responds to the interests and needs of the community. Several advisory board members, who represent area healthcare facilities, indicated their support for the Web-enhanced CLT/MLT program option. The college has made the commitment to support this option with the needed budgetary requirements. The major need for additional funding is a contract with a Web-authoring platform which would provide the electronic means of delivering the course material and provide the technical support for faculty and students. Once this contract is negotiated and the college commits the needed funds, the CLT/MLT program faculty will move forward with course development and implementation for Web-enhanced delivery beginning fall 2002.

In order to ensure the same level of learning outcomes for the Web-enhanced option as is currently experienced on-campus, students will need to secure a clinical laboratory site in which they will be instructed to perform the required psychomotor course competencies. Designated clinical instructors will work one-on-one with the student to assure correct performance of quality control, procedures, and reporting of results. There will also be an on-campus face-to-face portion required for the laboratory sessions. Students will be required to come to campus one day per month to work with the course instructor. This will allow the student to acquire exposure to procedures that may not be available in the clinical site and to interact with the instructor and other students. Cognitive skills will be delivered by placing the course material on the Web via a Web-authoring platform. The course syllabus, competencies, unit objectives, topics for asynchronous discussion, and exams will be available to the student in a Web-enhanced environment.

Faculty support is described in the Best Practices document as an ongoing program of appropriate technical, design, and production support. The institution should also provide the orientation and training to help faculty become proficient in the uses of the program's technologies including changes in course design and management. Other issues to be reviewed include faculty workload, compensation, ownership of intellectual property, and professional evaluation processes.8 An administrative decision has been made to support a Web-enhanced option, so the CLT/MLT faculty at SCCC has begun to modify the current course material to accommodate Web-enhanced delivery. The faculty will also participate in a training workshop sponsored by the selected Web-authoring platform to obtain technical skills to enable them to become proficient in this type of course delivery.

Student support should include an institutional commitment for continuation of the program to enable all students to complete the degree in the recommended timeframe. The institution should also provide students with advising, an on-line application process, placement testing, on-line enrollment, and access to financial aid, bookstore services, and library resources. The students will also require ongoing technical support 24-hours per day.8 SCCC currently is able to provide students with Web-based enrollment, bookstore services, and library resources. The contract with a designated Web-authoring platform will include 24-hour technical service for the student.

The assessment of student learning and evaluation of program effectiveness will be conducted through methods such as achievement of course competencies, written examinations, student attitude assessment to determine affective behavior, evaluation of student skills utilizing the CLT/MLT occupational profile and career development skills profile, and graduate performance on an external national certification exam.9 Course evaluations completed by the student will also be considered in the evaluation of program effectiveness. The CLT/MLT program will continue to participate in an external accreditation process with the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS) by submitting a self study document and hosting a site visit.
CURRENT DISTANCE LEARNING PROGRAMS
This researcher has identified eight CLS programs that offer Web-enhanced options. These programs are listed in Table 1. Of these eight programs, St Petersburg College (SPC) and Weber State University (WSU) have just completed the first year of the on-line option at the CLT/MLT level, with George Washington University Medical Center (GWUMC) offering the on-line option at the CLT/MLT level beginning fall 2002. The other programs have conducted a distance technology delivery method for over one year.

In order to identify these programs, the researcher posted a request for information via a clinical laboratory educators list serve. The program officials who responded to the request were then contacted via email and telephone. They were asked to complete a survey tool designed to collect data targeted at program format, on-line admission requirements, credit hour requirements, program costs, student costs, faculty workload recording, and student performance comparing on-campus versus Web-enhanced delivery. Of the eight programs identified, seven program officials responded to the survey for an 87.5% participation rate. These Web-enhanced programs offer degrees at the CLT/MLT associate degree level, the CLS/MT bachelor degree level, and the CLS/MT master degree level.

SURVEY ANALYSIS
The Web-enhanced programs utilize Web-based instruction as their primary means of delivery of the cognitive skills. Additional means of instruction include course material on compact disc (CD), video conferencing, conference calls, on-campus student laboratory sessions, asynchronous discussions, and chat rooms. The lecture portion of each course usually contains lecture notes in a PowerPoint format, unit outlines, and unit objectives. The Web-authoring platforms that are utilized include eCollege, WebCT, Blackboard, Prometheus, and WSU’s eCampus. Student examinations are administered by paper/pencil either on-campus or off-campus in a proctored setting or via computer assisted testing.

Students obtain the necessary skills by performing clinical laboratory procedures in various methods. These include attending on-campus student laboratories, performing laboratory procedures where the student is employed, receiving credit for previous work experience, and receiving credit for meeting psychomotor competencies as documented by an employer. In addition to the course associated laboratory sessions, students are also required to complete a clinical rotation or clinical projects to fulfill program requirements.

Fifty percent of the programs surveyed provide the option of a shortened clinical rotation contact hour requirement if the student is already employed in a clinical laboratory.

Web-enhanced CLT/MLT program admission requirements include grade point average, references, a computer with minimum technical requirements as identified by each institution, reliable Internet access, and a clinical laboratory in which to perform student training. WSU requires the online student to be employed in or sponsored by a clinical laboratory. The other responding programs indicated that the student or program officials must secure a cooperative clinical laboratory or develop a clinical affiliation agreement with a laboratory in the same geographical area as the student. The credit hour requirements for completion of a Web-enhanced CLT/MLT associate degree varies from 71 credit hours to 76 credit hours.

There are five Web-enhanced CLS/MT programs that allow articulation from the CLT/MLT to the CLS/MT. These programs include the WSU, the University of North Dakota (UND), Winston-Salem State University (WSSU), GWUMC, and the University of Arkansas for Medical Sciences (UAMS). Students making application for admission to these programs must possess an associate degree as a CLT/MLT with a minimum of 64 credit hours. The UAMS and WSU require national certification as a CLT/MLT. WSU and WSSU also require a minimum of three years work experience. GWUMC accepts CLT/MLT to CLS/MT candidates as long as they are graduates of an accredited CLT/MLT program. The applicants may be graduates of a civilian program or military CLT/MLT graduates who do not hold an AD but do have 60 or more credit hours on their transcript. The total credit hour requirements for the completion of a Web-enhanced CLS/MT BS varies from 120 credit hours to 129 credit hours.

Of the programs that responded to the survey questions pertaining to program start up costs, 75% indicated costs related to equipment, faculty course development stipends, and technology. Start up costs ranged from $3,000 to over $25,000. Equipment included video technology, microscope camera, digital camera, and CD burners. Faculty stipends for developing courses for the Web-enhanced format varied from no additional wages to $1,200 per credit hour. An additional cost for Web-enhanced delivery is a contract fee for the Web-authoring platform. Six of the programs surveyed indicated that program start up costs have been offset by the increased number of students enrolled in the Web-enhanced option.

REPORTS AND REVIEWS
Table 1. Current on-line CLT/MLT and CLS/MT programs

<table>
<thead>
<tr>
<th>Institution</th>
<th>Contact Information</th>
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In addition to tuition and fees, students enrolled in Web-enhanced courses will incur a $20 to $50 per credit hour or per course technology fee. Other costs to students may include upgrading to a computer that will meet Web-enhanced program requirements and maintaining a reliable Internet service connection. The UND requires a $500 per semester program fee. Students enrolled at UND may purchase course material on CD for $100 per course. As of fall 2002, WSU will charge a one time on-line program application fee of $150.

Survey respondents indicated that faculty workload recording is conducted by various methods. However, the majority indicated that faculty workload is based on credit hours taught per semester and that faculty workload for the Web-enhanced courses is equal to the traditional on-campus courses. WSU provides an on-line supplemental salary which is determined by a specific dollar amount per student per credit hour. All but one of the programs that currently have a Web-enhanced option have increased the number of on-campus and/or adjunct faculty to handle the increased student enrollment.

The final questions of the survey asked program officials to compare the performance of the traditional on-campus student to the Web-enhanced student. A small number of the respondents indicated that these questions were difficult to answer as they felt that these groups of students were entirely different. The on-campus student is the traditional, recent high school graduate with little or no work experience in the clinical laboratory. The Web-enhanced student is older, more committed to obtaining a degree, and often has work experience as a phlebotomist, clinical laboratory assistant, or CLT/MLT. Fifty-seven percent of the respondents believe that the majority of the students enrolled via a Web-enhanced course perform better than the on-campus students. However, attrition rates are higher in the first or second semester for the Web-enhanced students. The UND, WSSU, and the UAMS indicated that their graduates of distance education programs scored higher on a national certification exam. Barton County Community College (BCCC), along with UND, WSSU, and UAMS responded that the level of employability of graduates of the distance education program format equals that of the on-campus graduate.

Because 50% of the survey respondents indicated that their programs are still in the first one to two years of offering the on-line format, there are not enough data at this point to provide specific statistics regarding on-campus versus on-line student academic performance, laboratory performance, certification examination performance, and average time of on-line program completion. This author would propose that a follow up study of these on-line programs in the year 2007 would allow for more specific data regarding on-campus versus on-line overall student performance.

SUMMARY
There are a handful of CLT/MLT and CLS/MT programs that are leading the future trend of distance education via the Web and distance technology. With the advent of Web-enhanced learning and the severe clinical laboratory professional shortage, initiating a Web-enhanced program format at SCC should be feasible. Preliminary data indicate that there is an initial cost to the institution to implement the Web-enhanced format but that student enrollment increases offset this expense and make this a viable option. Providing degree options for laboratory personnel via distance education is important to students and current clinical laboratory employees who desire an advanced degree but cannot terminate their employment or relocate. Graduates of the Web-enhanced programs perform at the same level as, or better than, on-campus graduates on national certification exams and with regard to their entry level laboratory competencies.

ACKNOWLEDGEMENT
Sincere appreciation is extended to Leonard Bunselmeyer, Valerie Polansky, Kathryn Newton, A. Wayne Bruce, Donna Leach, Carol Smith, and Cherry Childs for providing individual program information.

REFERENCES
FOCUS: TRANSFUSION RISKS

Transfusion Risks: Transmission of Viral, Bacterial, or Parasitic Agents

MICHELLE WRIGHT-KANUTH, LINDA A SMITH

The transmission of viral, bacterial, or parasitic agents has been identified as one of the potential risks to an individual receiving a blood transfusion. For some well-recognized agents such as HIV or Hepatitis B, testing for antibody and the presence of viral nucleic acids in the donor has significantly reduced the risk of transmission. With newly identified viral agents whose transfusion-transmission potential is unknown, the determination of risk and the development of diagnostic tests represent complex tasks. For parasitic agents such as those that cause Chagas' disease or babesiosis, there are no serologic tests available. Chronic parasitemia with few circulating organisms makes identification of at-risk donors difficult and donor history questions are the sole means of identifying at-risk donors. Bacterial contamination of blood components is more problematic because of the varied ways in which bacteria can be introduced into the component and, until recently, the lack of effective and efficient ways to identify contaminated units. Small numbers of bacteria might not affect the recipient, yet a high level of bacterial contamination will cause significant morbidity and mortality. The articles in this FOCUS section discuss the viral, bacterial, and parasitic agents that are associated with transfusion transmission including their prevalence, current methods of testing, and the addition of tests or changes in testing requirements for each group of organisms.

LEARNING OBJECTIVES
After completion of the FOCUS Section, the learner will be able to:
1. List the viruses for which the U.S. blood supply is currently tested.
2. List virulent transfusion-transmitted viruses for which additional donor testing may soon be required.
3. Discuss the rationale for performing additional viral testing on donor units.
4. Discuss the effects of NAT testing on the incidence of transfusion-transmitted HIV and HCV.
5. Differentiate between viruses that are inactivated by solvent-detergent treatment and those that are not.
6. Identify and briefly explain three methods of viral inactivation that can be used for FFP.
7. Identify the major transfusion-transmitted parasites.
8. Identify the transfusion-transmitted parasite that is endemic in the U.S. and the geographical areas in which donors show the highest seroprevalence of the organism.
9. Describe current methods to prevent transmission of each of the parasites.
10. Explain the major reasons for concern with the transfusion-transmission of Trypanosoma cruzi in the U.S.
11. Identify the most common organisms that contaminate units of red cells and those that contaminate platelets.
12. Describe the primary methods to detect bacterial contamination in platelet units.
13. Describe the underlying cause of fatality in patients who receive units of blood contaminated with Yersinia enterocolitica.
14. Compare the level of bacterial contamination that can be detected with microscopy or automated methods.
15. Describe several methods by which the entry of bacteria into the unit can be decreased.
16. Explain why the use of leukoreduced units or apheresis (single donor) platelets will help reduce bacterial contamination.
17. Identify the major use of psoralen compounds in platelet units and the underlying mechanism of action.
FOCUS: TRANSFUSION RISKS

Transfusion-Transmitted Viruses

MICHELLE S WRIGHT-KANUTH, LINDA A SMITH

ABBREVIATIONS: AABB = American Association of Blood Banks; ALT = alanine aminotransferase; AMT = aminomethyl trimethyl psoralen; anti-H Bc = anti-hepatitis B core antigen; BPV = bovine papillomavirus; BSE = bovine spongiform encephalopathy; CDC = Centers for Disease Control; CJD = Creutzfeldt-Jakob disease; CMV = cytomegalovirus; DMMB = dimethymethylene blue; EBV = Epstein-Barr virus; FDA = Food and Drug Administration; FRALE = tangible anchor length effectors; GBV-C = hepatitis GB virus; GGT = gamma glutamyl transferase; H Av = hepatitis A virus; HbsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HGV = hepatitis G virus; H H = human herpes virus; HIV = human immunodeficiency virus; HPV = human papillomavirus; HTLV = human T-cell lymphotropic virus I and II; NAT = nucleic acid testing; NIH = National Institutes of Health; REDS = Retrovirus Epidemiology Donor Study; SD-D = solvent-detergent process; SD-FFP = solvent-detergent fresh frozen plasma; SEN-V = SEN virus; T NBP = tri (n-butyl) phosphate; TTV = transfusion-transmitted virus; UVA = ultraviolet A; vCJD = variant Creutzfeldt-Jakob disease; WNV = West Nile virus.

INDEX TERMS: blood testing; transfusion.

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Michelle S Wright-Kanuth and Linda A Smith are the Focus Transfusion Risks guest editors.

Focus Continuing Education Credit: see pages 246 to 248 for learning objectives, test questions, and application form.

The number and variety of viruses that have been proven to be transmitted by the transfusion of blood and blood products continue to increase. Among the viruses that are familiar to the clinical laboratory scientist are those for which the blood supply is currently tested: human immunodeficiency virus human immunodeficiency virus 1/2 (HIV 1/2), human T-cell lymphotropic virus (HTLV) I and II, hepatitis C virus (HCV), hepatitis B virus (HBV), and, in some cases, cytomegalovirus (CMV). The transmission of West Nile virus (WNV) by both transfusion and transplantation has recently emerged as a concern. Donor testing for WNV was implemented as soon as a test for its detection was approved in the summer of 2003. Additionally, the testing of donor units for hepatitis A virus (HAV) and parvovirus B19 is under review and is likely to be implemented in the near future. Donors who have either resided or traveled in the United Kingdom (UK) or continental Europe for an accumulation of three months between the years 1980 and 1996, or injected bovine insulin from the UK, are deferred from blood donation in the United States (U.S.) because they are considered to be at risk for variant Creutzfeldt-Jakob Disease (vCJD), a form of spongiform encephalopathy that may be transfusion-transmissible.

Several other viruses are either transfusion-transmitted or suspected of being so. SEN virus (SEN-V) has recently been investigated in transfusion-associated hepatitis. Transfusion-transmitted virus (TTV), an aptly named viral agent, is prevalent in the donor population and may be associated with chronic alanine aminotransferase (ALT) elevation in some individuals. Transfusion has been implicated in the tran-
mission of Epstein-Barr virus (EBV) in a case of possible transfusion-transmitted infectious mononucleosis. EBV has also been associated with posttransfusion hepatitis. Several human herpes viruses (H HV-6, H HV-7 and H HV-8) have been studied as to possible parenteral transmission. A virus known variously as hepatitis G virus (GBV-C) and hepatitis G virus (H GV) has also been determined to be transfusion-transmitted, although it does not appear to cause any disease state and is not associated with hepatitis, as previously thought. Bovine papillomavirus (BPV) has been shown to be transfusion-transmitted in cattle, raising questions about human papillomavirus (HPV).

The number and variety of the viruses that have been associated with blood transfusion require the immunohematologist to be familiar with the viruses and the methods of detection and/or inactivation that may be employed to protect the transfused patient from viral transmission. All of the viruses listed above will be reviewed in this article, along with their clinical significance, testing being performed to detect them, and treatments of blood components to inactivate them.

**FAMILIAR TRANSFUSION-TRANSMITTED VIRUSES**

U.S. donors are currently screened for infection with H CV, HBV, HIV, and HTLV-I and –II, and in some instances, CMV. H CV is a single-stranded RNA virus of the Flaviviridae family. H CV transmission in the U.S. appears to be primarily parenteral, with sexual transmission a less efficient form of transmission. Blood transfusion accounted for a large number of cases identified prior to the implementation of donor screening for the virus. Greater than 90% of the cases of non-A, non-B hepatitis caused by blood transfusion were due to the transmission of H CV prior to 1990. Between 1990 and 1998, anti-H CV testing was used alone to detect infected donors. As a result of this testing, the incidence of transfusion-transmitted HBV was reduced to approximately 1 in 103,000. In 1998, nucleic acid testing (NAT) for H CV RNA was implemented to screen all blood donors in the U.S. Since that time, both anti-H CV and NAT testing for H CV RNA have been used in the U.S. and the current risk of transmission per unit of blood donated has been estimated to be 1 in 1,600,000.

H BV is a member of the Lentivirus subfamily of the Retroviridae. As with H CV, the transmission is primarily sexual and parenteral, although maternal-child transmission is also possible. The implementation of H IV antibody testing of donors in 1985 decreased the number of transfusion-transmitted cases from 714 reported in 1984 to about five cases per year from 1985 to 1990. In 1996, the Retrovirus Epidemiology Donor Study (REDS) estimated that 1 in 63,000 donations in the U.S. may transmit HBV to the recipients because the donor, although HBV positive, was in the pre-H BsAg positive ‘window period’ when the donation was made. Therefore, even with donor testing for HBsAg and anti-H Bc in place, some transfusion-transmission of HBV was unavoidable. Source plasma manufacturers have recently implemented NAT testing for HBV DNA, as have blood donor facilities in Germany and Japan. Although there is considerable discussion regarding the need to do so, it seems likely that HBV DNA NAT testing will be adopted in the U.S. soon. Examples of the conflicting reports include a study by Otake and Nishioka in Japan that showed the detection of HBV DNA by NAT method in seven units that were tested as a part of minipools of 50 donors. All seven of these donor units were negative for HBsAg, H BcAg, anti-H Bs, and anti-H Bc by the Auszyme II enzyme immunoassay method. However, the U.S. studies cited by Sacher and coworkers indicated that HBV is present in very small amounts during the window period. Studies in the U.S. that have evaluated several HBsAg methods against several HBV DNA NAT methods appear to show that, while currently FDA licensed methods for NAT testing will detect infected donors that are not detected by currently licensed HBsAg methods, there are HBsAg methods under FDA review (though not yet licensed) that are as good as the NAT methods available.

H IV is a member of the Lentivirus subfamily of the Retroviridae. As with H CV, the transmission is primarily sexual and parenteral, although maternal-child transmission is also possible. The implementation of HIV antibody testing of donors in 1985 decreased the number of transfusion-transmitted cases from 714 reported in 1984 to about five cases per year from 1985 to 1990. In 1996 the risk per donor was calculated to be 1 in 493,000. In 1996, HIV p24 antigen testing was added to anti-H IV testing, further reducing the risk of transmission to 1 in 641,000. The cumulative number of HIV cases attributable to transfusion-transmission prior to June of 1999 was 8,793. Of these cases, only 40 were traced to blood transfusions given after
the implementation of H IV antibody testing in March of 1985. The few cases due to screening failure at this time resulted from donations from infected individuals with viremia that were obtained prior to the development of detectable antibody responses, the so-called ‘window period’. The development and subsequent implementation of p24 testing reduced the ‘window period’ from 45 days in 1990 to about 16 days, due to the ability to detect circulating viral antigen earlier than detection of antibody. However, a few cases of transfusion-transmitted H IV still occurred due to donation during the ‘window period’.

H IV type-1 RNA has been detected in the U.S. blood donor population by NAT since 1998, further reducing the window period to about 11 days. Testing for H IV-1 RNA has replaced testing for p24 in U.S. donor population. Due to cost concerns, pools of plasma from 16 to 24 donations, rather than individual donor samples, are currently NAT tested for H IV-1 RNA. A case reported in 2003 documented the transmission of H IV in a unit of red cells that had screened negative for H IV RNA using the minipool NAT screening currently employed. Further reductions in the window period may be possible by testing each unit for H IV-1 RNA separately. As a result of using minipool NAT testing in conjunction with anti-H IV 1/2 testing, the incidence of transfusion-transmitted H IV has been reduced to approximately 1/1,800,000. It is not possible, however, to detect every infected unit due to decreased viral load in some individuals, viral mutation, and the failure of some infected individuals to form antibody.

H TLV-I and H TLV-II were the first of the human retroviruses to be identified as transfusion-transmitted. These viruses have been implicated in T-cell leukemias, polyomyositis, arthritis, and myelopathies and are also transmitted through sexual, IV drug abuse, and perinatal mother to child methods. The prevalence of antibodies to H TLV was shown to be 0.025% in the U.S. donor population in 1988, the year donor testing was introduced for antibodies to H TLV-I. Blood containing H TLV that has been stored for more than 14 days, as well as non-cellular blood components, appears to be non-infectious. Using current tests that detect antibody specific to both H TLV-I and H TLV-II, the risk of H TLV transmission is estimated by Schreiber and colleagues to be approximately 1 in 641,000 units. However, serious clinical disease is seen in approximately 5% of individuals infected with H TLV, indicating that the actual risk of disease is considerably lower.

CMV is a DNA virus of the Herpesviridae family that is easily transmitted by transfusion, but is also spread through the saliva and the semen. It is usually not significant in immunocompetent individuals, but can be of serious consequence, and sometimes fatal, in neonates, infants, and immunosuppressed patients. Organ and tissue transplant patients are at risk not only for transfusion-transmitted, but also for transplantation-transmitted CMV. No routine screening of the blood donor population is currently employed. The virus is leukocyte-associated and leukoreduction of blood products is effective in reducing transmission. The presence of less than 107 leukocytes per unit achieved through leukoreduction makes CMV transmission less likely. Transfusion-transmitted CMV is currently controlled in susceptible populations through either leukoreduction of the blood product or the use of products negative for anti-CMV. The transmission of CMV when these types of products are used can occur, however, with rates reported as high as 2%. One cause for such transmission may be plasma viremia during the first four weeks of infection (the ‘window period’). Drew and colleagues, in a study of a CMV DNA PCR assay, showed that plasma CMV DNA was detected in two instances before the development of CMV antibody. There are conflicting reports, though, about the efficacy of testing for CMV DNA. A large cross-sectional study of U.S. blood donors showed that CMV DNA was not detected in any of 514 samples that were negative for anti-CMV, nor was CMV DNA detected in any of 70 samples that had discrepant CMV serology results. The authors of that study concluded that the addition of CMV DNA PCR to donor screening would not increase detection of CMV positive donors over screening for anti-CMV.

OTHER VIRULENT TRANSFUSION-TRANSMITTED VIRUSES

Several other virulent viruses are known to be transmitted through transfusion. These include EBV, H HVs, H AV, and WN V. The SEN virus and vCJD prion may also be among them. EBV, H HV-6, H HV-7, and H HV-8 are all double stranded DNA viruses of the family Herpesviridae. EBV is the causative agent of infectious mononucleosis and Burkitt’s lymphoma, and has been implicated in post-transfusion hepatitis. A case in France of transfusion-transmitted infectious mononucleosis due to EBV was recently documented. Although EBV is primarily spread through the saliva and most individuals have been infected by adulthood, transfusion-transmission has been problematic in a few cases, with the development in the recipient of subsequent infectious mononucleosis or post-transfusion hepatitis linked to a donor positive for EBV. In immunocompetent individu-
als, transfusion-transmitted EBV is asymptomatic; however, immunocompromised individuals who are seronegative may be at-risk for disease. Since EBV infects the B lymphocytes, leukoreduction may afford some protection to those at-risk. HAV-6 and HAV-7 cause exanthem subitum (roseola), a condition marked by high fever and convulsions in children. HAV-6 has also been associated with hepatitis and mononucleosis in adults. Luppi reports that HAV-6 is prevalent in the leukocytes of blood donors, as does Kozireva. Transmission of disease caused by HAV-6 through transfusion has yet to be established, however. Seropositivity in adults is very high and no current recommendations exist for protection of seronegative transfusion recipients. HAV-7 DNA was found in the plasma of 10.6% of Latvia blood donors, although there are no current indications of transfusion-transmitted disease.

HAV-8 is associated with Kaposi's sarcoma, Castleman's disease, and other B-cell tumors. HAV-8 transmission through organ transplantation has been demonstrated, although the primary route of transmission appears to be sexual. A study in Tanzania showed that HAV-8 DNA can be detected in serum of the blood donor population. Studies in both France and the U.S. showed that anti-HAV-8 is detectable in the serum of blood donors, with a seroprevalence in Texas blood donors of 15%. A study of 100 donors in the Houston, Texas blood donor population failed to find HAV-8 DNA in any sample, although 25% of the donors had antibody to HAV-8. O perskalski showed that in a group of 14 recipients of documented HAV-8 positive blood, none were seropositive 19 months after the transfusion.

HAV is a single-stranded RNA virus belonging to the family Picornaviridae. It has traditionally been considered to be transmitted solely by the fecal-oral route and not by transfusion. However, in 1994, Vermilyen and Peerlinck reported hepatitis A in hemophiliacs that was shown to have been transmitted through contaminated factor VIII concentrates. As a result of this and other similar reports, U.S. plasma fractionators implemented NAT testing for HAV during 2001. Although blood-borne transmission of HAV is apparently rare, with an incidence of approximately 1 in 1,000,000, transfusion-transmitted hepatitis A does occur. Two cases of transfusion-transmitted hepatitis A from a single whole-blood donation that was prestorage leukoreduced were reported by Diwan in 2003. The donor was diagnosed with hepatitis A 18 days after making the donation. There is evidence that HAV viremia is present for up to 30 days prior to the onset of symptoms. Therefore, calls have been made for the institution of HAV DNA NAT testing of blood donors in the U.S. Implementation of such NAT testing is likely in the near future.

WNV was first recognized in North America in 1999. It is a single-stranded RNA virus member of the Flaviviridae family. Major vectors are mosquitoes and the primary hosts are birds, although humans and other mammals can be infected when bitten by infected mosquitoes. The virus is fatal in 1 of 1,000 cases in humans. Several cases each of possible transmission by transfusion and transplantation have been reported. Three cases of donor to recipient transmission are well documented by the Centers for Disease Control (CDC). The first case was a multiorgan donor that received more than 60 units of blood products during treatment prior to her death. Four separate recipients of her organs all developed WNV disease through transplantation; none of the recipients had other possible exposures to WNV. In a second case, a red cell recipient developed WNV and the virus was detected in the plasma from the same donation. The third case involved a donor from whom two recipients contracted WNV. A postpartum recipient of red blood cells from the donor with the disease developed WNV. A platelet concentrate made from the same donation as the red cells was later found to be positive for WNV. Based on these three separate, clearly documented cases, as well as several others, the CDC has called for the development of screening assays, for WNV.

The Canadian Blood Services plans to implement WNV testing of donors by summer 2003. A major effort was made to develop and implement NAT testing for WNV RNA on donor units in the U.S. by the summer of 2003.

Parvovirus B19, a single stranded DNA virus of the Parvoviridae family, is probably best known as the cause of fifth disease, also known as erythema infectiosum, in children. Infection may also cause fulminant hepatitis even in immunocompetent individuals. This virus is also associated with fetal infection and stillbirth when pregnant women are infected. Infection of immunocompromised individuals may result in red cell aplasia. Hayakawa and colleagues reported a case in which parvovirus B19 was transmitted by intravenous immune globulin. Three cases of transfusion-transmitted B19 were documented by Azzi and others in 1999. A group of French investigators described the presence of antibodies to parvovirus B19 in hemophiliacs. With the detection of parvovirus B19 DNA in plasma pools and derivatives, the implementation of NAT testing of U.S. blood donors seems likely in the near future.

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FOCUS: TRANSFUSION RISKS

Creutzfeld-Jacob disease (CJD) has been known for many years as a possible transfusion-transmitted degenerative disease. The classical form of CJD is familial; 10% to 15% of the time. The other 85% to 90% of cases are sporadic. The infection is thought to be caused by a prion, which is a protein particle. Classic CJD is very slowly progressive, with symptoms developing many years after infection. Once the symptoms develop, the disease then progresses rapidly from dementia and lack of motor skills to death. The incidence of CJD is extremely low, but donors have been asked about family history of CJD due to the demonstration of the prion in human leukocytes and documented transmission of CJD through injection of human growth hormone. The donor history questionnaire asks the donor to reveal any injection of human growth hormone; donors who have received it are indefinitely deferred. The recent development of a new variant form of this disease has sparked increased concern.

vCJD is a fatal degenerative disease first reported in England in 1996. The vCJD form is fatal very rapidly from the onset of the infection and has a much higher incidence than CJD. Approximately 140 cases have been reported worldwide as of the end of 2002. vCJD appears to be caused by the same infectious agent as bovine spongiform encephalopathy (BSE). A study in the UK showed that it is possible to transmit BSE to a sheep by transfusion with whole blood from another sheep. The donor sheep was in the preclinical phase of an experimentally induced BSE infection and appeared healthy when the donation was taken. Several other animal models have been used to show that transmission of related spongiform encephalopathies can be transmitted by transfusion; however, no human cases of transfusion-transmitted vCJD have been documented. No testing is yet available to detect the causative agent, possibly a prion, in the blood of asymptomatic individuals. As previously noted, significant deferral mechanisms, including deferral of any donor who has traveled extensively or lived in continental Europe, are in place to try and prevent transmission.

APPARENTLY AVIRULENT TRANSFUSION-TRANSMITTED VIRUSES

SEN-V has been reported as a single-stranded, circular DNA virus. Standard sequencing studies have shown the existence of eight different genotypes of SEN-V, designated SEN-V A through H. SEN-V is demonstrable through NAT testing; however, no test for SEN-V antibody is currently available. A study in cooperation with the National Institutes of Health (NIH) showed that 92% (11 of 12) of patients suffering from transfusion-associated non-A to E hepatitis were infected with SEN-V after transfusion, compared with 24% (55 of 225) of recipients followed identically who did not develop hepatitis. Also shown in the same study was that the incidence of SEN-V infection after transfusion was 30% (86 of 286) while non-transfused controls had an incidence of 3% (3 of 97) and the prevalence of SEN-V in 436 volunteer donors was 1.8%. Since the majority of infected transfusion recipients did not develop hepatitis, no real cause and effect of SEN-V in this regard has been proven. In a study by Pirovano, a similar incidence of SEN-V infection between HIV and HCV patients was shown at 45% and 46% respectively. It was found in this study that the prevalence of SEN-V infection was significantly higher (p<0.0001) among IV drug user than non-IV drug user HIV-positive individuals. A study of several SEN-V positive transfusion recipients by Ball demonstrated sequence homology between donor and recipient, showing transfusion-transmission. Since parenteral transmission is apparent, SEN-V has been studied further for implication in post-transfusion hepatitis. SEN-V viremic individuals on hemodialysis were studied by two different groups, one in Germany and one in Japan. Each showed that, while SEN-V infection is common in hemodialysis patients, none of those studied by either group of researchers developed hepatitis without co-infection with HCV. Also, both groups showed that SEN-V infection was not associated with the amount of transfusion. Currently, it appears that, while SEN-V is transfusion-transmitted, there is no clear association with production of disease.

HGV, also known as GBV-C, is an RNA virus of the Flaviviridae family. HGV has a 29% amino acid homology with another Flavivirus, HCV. Several studies have documented that transfusion-transmission and coinfection with HCV is common. The virus has been found in from 1% to 2% of the donor population in the U.S. In Germany, both high levels of antibody to HGV (17.5%) and high levels of viremia (19.6%) in hemodialysis patients were seen. These patients had a significant (p<0.05) increase in incidence of HGV infection when more than five transfusions had been received. High frequencies of HGV were also reported in Japanese blood donors, with 15.8% of 203 donors tested being positive for HGV RNA. The site of HGV replication does not appear to be in hepatic cells, since HGV is not hepatotropic, but may occur in the mononuclear cells of the spleen or bone marrow. There is a documented absence of correlation between infection with HGV and clinically significant liver disease. A study in Italy showed that, while HGV infection is common among kidney transplant patients, chronic liver disease was not increased in these pa-
tients as compared to kidney transplant patients that were HGV negative. Studies of patients with acute non-A through E hepatitis in Sicily demonstrated that only 35% (19 of 54) were infected with HGV and none of the HGV infected patients progressed to chronic liver disease. No association between HGV positivity and ALT or gamma glutamyl transferase (GGT) elevations were seen in a study of peritoneal dialysis patients in Italy. In one study, HGV or GBV-C RNA was the only viral marker seen in three patients with a mild form of hepatitis. These three patients, out of 79 studied, had only slightly elevated ALT levels. However, a pediatric patient in Greece who developed fulminant hepatic failure was shown to be positive for HGV RNA and negative for all other known hepatitis viruses. Also, a study in China showed that 52 patients infected with HGV had no other hepatitis viral markers present, yet showed pathological liver changes. Thus, these results indicate that the pathogenicity of HGV is unclear at present and more research is necessary to determine whether or not this virus is a causative agent of post-transfusion hepatitis. There appears to be no evidence that HGV causes any other clinical disease state.

TTV is a single-stranded DNA virus of the Circoviridae family. It was initially reported in patients with posttransfusion hepatitis of unknown etiology. The virus appears to be prevalent in the healthy population, with a rate of 65% (36 of 55) in healthy, previously non-transfused children studied in the Washington DC area. TTV seems to be transmitted primarily by the oral-fecal route in children under five years of age. The virus is transfusion-transmissible; however, this route is not a major source of the virus epidemiologically. No specific pathogenicity of this virus has been described although an association with posttransfusion hepatitis has been explored. In a Chinese study of the prevalence of TTV in blood donors, 36 out of 340 (10.6%) blood donors tested were positive for TTV. Prior to transfusion 11 of 130 (8.5%) recipients of these units were positive for TTV. Of the 119 recipients of the blood that were TTV negative prior to transfusion, 18 were positive after transfusion; however, only one of the corresponding donors was reported to be TTV positive. The prevalence of TTV DNA in blood donors screened using a specific type of TTV primers is as high as 80%. TTV, therefore, appears to be prevalent in the healthy population without causing disease.

ANOTHER POSSIBILITY?

HPV may be transfusion-transmissible. An interesting report from Brazil concerns the transfusion transmission of BPV in cattle. BPV is a virus with similarities to HPV. The documentation of the transmission of BPV both through blood transfusion and vertically may be of significance. Although only one paper regarding such transmission can be found, it raises interesting possibilities. The vertical transmission of HPV has been suggested by Sedlacek and colleagues. HPV DNA has been demonstrated in the lymphocytes of women with cervical cancer. Whether or not this is an indication that we may find yet another transfusion-transmissible virus remains to be seen.

PREVENTION BY VIRAL INACTIVATION

Inactivation of pathogens in plasma products has been in use for several years. The solvent-detergent (S-D) process was initially licensed in 1985 for use in the manufacture of factor VIII concentrates. To use this process on fresh frozen plasma (FFP), pools of between 380 and 2500 ABO identical units are made after thawing and the plasma is filtered to remove cell debris. Treatment with a 1% tri (n-butyl) phosphate (TNBP) solution as solvent, containing Triton X, Tween 80, or sodium cholate detergent is commonly used. The treatment is performed at 30 °C for four hours. The detergent disrupts viral lipid envelopes. After treatment the solvent must be removed. The plasma is then sterile filtered and frozen in 200 mL aliquots. HCV, HBV, HTLV-I and II, HIV, HGV, and WNV are all enveloped viruses and most have been shown to be reduced considerably by S-D treatment. Many transfusion-transmitted viruses, such as HAV, HPV, and parvovirus B19, are either non-enveloped or their envelopes have not been detected, and have not been shown to be removed by S-D treatment.

Methylene blue treatment of FFP to inactivate viruses was first described in 1991. This phenothiazine dye has been shown to inactivate retroviruses, herpes viruses, HCV, HBV, and WNV in both plasma and red cells. Leukocytes are first removed from the plasma, either by filtration or freezing and thawing. After leukocyte removal and the addition of methylene blue to a concentration of 1 μM, the units of plasma are exposed to white light. This photoinactivation process is performed on single units, as opposed to large pools used for S-D FFP preparation. Methylene blue and other photodynamic inactivators have also been studied for use in platelets, but appear to damage the platelets in the process. In red cell concentrates, photodynamic processes tested thus far cause red cell damage. A phenothiazine compound, dimethylmethylene blue (DMMB) is comparable or better than methylene blue for inactivation of viruses but does not cause as much red cell damage.
Psoralens may also be used to inactivate viruses in FFP and in platelet concentrates. The psoralens, such as aminomethyl trimethyl psoralen (AMT) and S-59, added to the plasma will intercalate with nucleic acid and, when then exposed to ultraviolet A (UVA) light for three or four minutes, will form a stable photoadduct between nucleic acids strands and pyrimidine bases. After the procedure is completed, the residual S-59 and unbound products must be removed. This process can be used for single or jumbo units of plasma for FFP. HIV, HCV, and several other viruses have been shown to be inactivated by this process. S-59 may have an advantage in that prefiltration and freeze-thaw steps to remove leukocytes are not needed and bacterial inactivation has also been shown to occur.\textsuperscript{85,87}

Pasteurization has also been used successfully to inactivate viruses in plasma products such as IVIG and coagulation factors, but not in FFP. All lipid-enveloped viruses are inactivated as well as several non-enveloped viruses. Time and temperature, as well as protein concentrations must be monitored when this method is used.\textsuperscript{86}

Caprylates, naturally occurring fatty acids found in animal and vegetable fats, have also been studied for virus inactivation in IVIG and albumin plasma products. Caprylate is added to facilitate the precipitation of unneeded proteins at 2 °C to 8 °C, then, by readjusting the concentration and raising the temperature to 25 °C, enveloped viruses were inactivated. When HIV-1, and virus models for HCV and herpes viruses were tested, caprylate inactivated them in IVIG products as well as S-D methods did.\textsuperscript{92}

Frangible anchor length effectors (FRALE) have also been developed.\textsuperscript{93} FRALEs are molecules that are activated by a shift in pH after they are added to packed red blood cells and then form covalent adducts with DNA and RNA. The compound tested by Cook and colleagues inactivated HIV, several other viruses and both gram-negative and gram-positive bacteria. Posttransfusion red cell recovery using FRALE-treated RBCs in both murine and canine models was comparable to posttransfusion recovery of untreated RBCs. Clinical trials are underway.\textsuperscript{85,93}

Other processes to remove infectious agents from immune globulin preparations include nanofiltration, which has been shown to remove prion material, antibody-coated parvoviruses, and antibody-coated enteroviruses. This process may show some promise in eliminating the non-enveloped viruses and prions from transfused products.\textsuperscript{94,95}

Transfusion-transmitted viruses, while increasingly being discovered and recognized, are also being tamed. New viruses emerge routinely, however, and require vigilance from the transfusion community. As demonstrated by the furor surrounding the advent of transfusion-transmitted HIV, the public demands safety of the blood supply approaching zero percent viral transmission. The blood banking community has accepted this challenge. New testing is constantly being implemented to insure an uninfected blood supply. New methods for treating blood components to inactivate viruses show promise in the fight against transmission, and FFP is routinely treated prior to distribution. Inactivation of viruses in platelet concentrates and packed red cells is on the horizon.

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FOCUS: TRANSFUSION RISKS


FOCUS: TRANSFUSION RISKS

Bacterial Contamination of Blood Components

LINDA A SMITH, MICHELLE S WRIGHT-KANUTH

ABBREVIATIONS: AABB = American Association of Blood Banks; BaCon = bacterial contamination associated with transfusion reactions; CFU = colony forming units; RBC = red blood cell; SHOT = serious hazards of transfusion; UVA = ultraviolet A light.

INDEX TERMS: bacterial contamination; transfusion contamination.

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Michelle S Wright-Kanuth and Linda A Smith are the Focus: Transfusion Risks guest editors.

Focus continuing Education Credit: see pages 246 to 248 for learning objectives, test questions, and application form.

As discussed in one of the companion articles, significant progress has been made in reducing the transfusion transmission of viral infections such as hepatitis B, hepatitis C, and HIV. Serologic testing of donors has decreased the risk for acquiring some viral diseases to less than one in a million. Now however, bacterial contamination of blood products has assumed a major role as a cause of morbidity and mortality in recipients of blood products. Although overall risk for acquiring a transfusion-transmitted bacterial infection is still relatively low, methods to detect infected donors are not available as they are for detection of virally-infected donors. Therefore, detection of bacterial contamination is more problematic and is usually recognized only when the recipient has a severe reaction following administration of the blood component. In fact, after acute hemolytic transfusion reactions, bacterial sepsis is the next most frequent cause of transfusion related fatalities.

In this article we will discuss the major organisms associated with bacterial contamination of blood components, studies of prevalence of bacterial contamination and transfusion associated sepsis, and the methods developed or in development to detect and/or prevent contamination.

BACTERIAL CONTAMINATION

Bacterial contamination of blood units occurs at any one of the following points: collection, processing, pooling of components, or transfusion. Infrequently, it may be due to transient bacteremia in the donor. High bacterial concentrations are often responsible for a serious septic or fatal reaction in the recipient with most of these reactions occurring in the elderly, neonates, or patients immunocompromised by illness or chemotherapy. If patients who receive contaminated units are on antimicrobial therapy, there is often decreased severity of the clinical reaction, making recognition more difficult. On the other hand, low levels of bacterial contamination in the transfused component may cause relatively mild symptoms, such as fever and chills, which resemble a febrile, non-hemolytic transfusion reaction.

It is widely recognized that the similarities in these mild symptoms has led to the underrecognition and underreporting of reactions due to bacterial contamination of blood products. Over the years, the literature has described case reports of one or more fatalities due to bacterial contamination of blood components or results from surveillance surveys designed to detect the prevalence of bacterial contamination and/or transfusion-associated bacterial sepsis in a single facility. Comparing these reported data or obtaining an overall incidence rate can be difficult due to different criteria for identification and reporting of a reaction, timing of cultures, and methods used to detect contamination. In the last few years however, there have been concerted efforts in several countries to obtain data about the overall prevalence of bacterial contamination.
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contamination in blood products and transfusion-related fatalities in a standardized manner.11-14 The English SHOT (Serious Hazards of Transfusion) project, the French Hemovigilance effort, and the U.S. BaCon study (Bacterial Contamination Associated with Transfusion Reactions) are ones that have received wide dissemination and will be discussed later in the article.11,14

Blood is an excellent growth medium but only a few bacterial species such as Yersinia, Serratia, and Pseudomonas grow well at 1 °C to 6 °C, the storage temperature for red blood cells (RBC). Most bacteria, however, grow well at 20 °C to 24 °C, the storage temperature for platelets which is why surveillance studies often show a higher rate of bacterial contamination for platelets than for RBC.1,15

Normal skin flora such as coagulase-negative Staphyloccocys, yeast, Streptococcus spp., Bacillus cereus, Propionibacterium acnes, or gram-negative bacilli that are attached to skin cells or colonized in sebaceous glands are introduced into the unit with the initial skin plug when the needle enters the arm during phlebotomy. When the platelet concentrate made from the blood is incubated at room temperature, these bacteria rapidly and can increase from as few as one to five colony forming units (CFU)/mL to greater than 1x10^8 CFU/mL during the five day shelf-life.1,15-17 Generally bacterial levels greater than 10^8 CFU/mL are associated with severe and often fatal outcomes.18

Although the FDA requires reporting fatalities related to transfusion, there is no requirement for reporting milder reactions. From 1976 to 1985 in the United States, there were 256 deaths directly related to transfusion and approximately 10% of these were traced to bacterial contamination of a blood product.3,4 In another report from the years 1986 to 1991, there were 182 transfusion-related deaths documented with 29 of those deaths due to bacterial contamination.19 Over the past several years in Canada there have been 11 severe reactions associated with bacterially-contaminated components; seven with platelet pools and four with RBC.20

ORGANISMS IN RBC COMPONENTS

The overall fatality rate as a result of contaminated RBC units is approximately one in every million units transfused.4,10,21 Yersinia enterocolitica, Serratia spp. and Pseudomonas spp. represent more than 50% of the implicated organisms that are reported to FDA, with Y. enterocolitica being the most frequently identified.5,9,22,23 Y. enterocolitica causes an acute enteritis characterized by fever, nausea, and diarrhea as well as a transient bacteremia associated with an asymptomatic period before or after the episode of enteritis.24 If a donor is drawn during this asymptomatic period, the phagocytized organism within the donor's white blood cells is released into the unit when the white cells disintegrate. Y. enterocolitica is capable of growth at 4 °C in the presence of iron and glucose and subsequently produces endotoxin. After an initial lag phase of 10 to 20 days the organism rapidly reproduces throughout the shelf-life of blood to concentrations of greater than 10^8 CFU/mL. Therefore the longer blood is stored, the more likely that the concentration of endotoxin will be high enough to induce sepsis.25,26

Between 1985 and 1996 there were 21 cases of sepsis due to Y. enterocolitica contaminated RBC, with a total of 12 deaths.24,26,28 Ten of these cases and five fatalities occurred between 1991 and 1996. When implicated donors were questioned in follow up interviews, several reported diarrheal episodes within a few weeks before or after donation; but other donors reported no symptoms. The first case of Y. enterocolitica contaminated platelets has been reported from a patient who received pooled platelets. The associated unit of RBCs, which had not been transfused, also grew Y. enterocolitica.29

The genus Serratia contains opportunistic organisms that grow in moist areas including the respiratory and gastrointestinal tract of animals, as well as the environment. They are associated with nosocomial infections such as urinary tract infections or wound infections. S. marcescens and S. liquefaciens have been isolated from blood components. These organisms are capable of growth over a wide range of temperatures including 4 °C, and like Y. enterocolitica, produce endotoxin. The organisms can adhere to plastic transfer bags and derive nutrition from carbon sources in the water soluble plastics of blood bags.30 In the last ten years, S. liquefaciens has gained increased prominence as a cause of transfusion-associated sepsis. Between 1992 and 1999 there were five cases of S. liquefaciens sepsis (80% fatality rate) – three from infusion of RBC, one from a platelet transfusion, and one from autologous blood.30,31,32 S. liquefaciens is a dark broth and can be isolated from the blood bag and culture of the recipient's blood confirmed a fatal case of sepsis in the United Kingdom.33 In the cases of RBC contamination, several of the contaminated units appeared hemolyzed or had an unusual dark color. Symptoms started after as little as 20 mL to 50 mL of blood were infused and recipients developed septic shock due to an increased endotoxin level.31
Even autologous blood carries a risk for bacterial contamination. Several patients who developed transfusion-transmitted \textit{Y. enterocolitica} infection had received autologous transfusions.\textsuperscript{23,33} In a Japanese study, the most common contaminating organism in autologous blood was coagulase-negative \textit{Staphylococcus} with the highest contamination rate found in intra-operative salvage units.\textsuperscript{34}

**ORGANISMS IN PLATELET CONCENTRATES**

The risk of a bacterially contaminated platelet transfusion is 50 to 250 times higher than risk of virally contaminated one.\textsuperscript{5} Because of the rapid growth of organisms at room temperature and increased risk of resultant sepsis in the patient, platelet storage is limited to five days. In most cases, the units implicated in bacterial sepsis are those that are four to five days old and have bacterial counts well in excess of $10^6$ CFU/mL.\textsuperscript{18,35,36} The causative organisms in platelet contamination are more varied than those found in RBC. They are primarily normal skin flora with coagulase-negative \textit{Staphylococcus} (usually \textit{S. epidermidis}), accounting for more than 50% of the isolates.\textsuperscript{18,35-38}

As previously mentioned, several fatal cases were due to organisms such as \textit{Y. enterocolitica} and \textit{S. liquefaciens} that are usually associated with contaminated RBC. Other organisms that have been reported in fatal sepsis cases due to infusion of contaminated platelets include: methicillin resistant \textit{S. aureus}, \textit{Clostridium perfringens}, \textit{S. epidermidis}, \textit{Salmonella enteriditis}, and \textit{S. marcescens}.\textsuperscript{35,39-43}

Most often however, transfusion of units contaminated with normal flora is not fatal but rather causes symptoms similar to those of a febrile, non-hemolytic transfusion reaction. The frequency of contamination of units is much higher than episodes of sepsis. Reports of incidence of bacterial contamination of platelets ranges widely with most estimating contamination as approximately 1 in 2,000 to 1 in 3,000 units, regardless of whether the source is single donor (apheresis) platelets or platelets made from whole blood.\textsuperscript{11,18,36,44,45} Risk for contamination in pooled platelets, because of the pooling, is higher than the risk in apheresis platelets.\textsuperscript{44} Septic reactions are estimated to occur in one-quarter to one-sixth of

<table>
<thead>
<tr>
<th>Study</th>
<th>Years</th>
<th>Total confirmed due to bacterial contamination</th>
<th>Components involved</th>
<th>Number of fatalities</th>
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<td>1996-1998</td>
<td>4</td>
<td>1 RBC</td>
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<td>3 platelet</td>
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<td>185</td>
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<td>23 random</td>
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<td>Bacthem\textsuperscript{11} (Case control)</td>
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<td>41</td>
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<td>BaCon\textsuperscript{12} (Voluntary)</td>
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contaminated transfusions with estimates for overall mortality as high as 26%.2,5

Several national studies have looked at the organisms involved in transfusion related incidents and the fatalities that occurred. Table 1 summarizes the number of incidents and fatalities reported. The SHOT study in England was a voluntary reporting study designed to track complications associated with blood transfusions.13 Clinicians responsible for transfusions reported 366 cases of complications between 1996 and 1998. Of these, 12 were confirmed as transfusion-transmitted infections, with four being bacterial in origin, seven viral, and one malarial. The three non-fatal bacterial infections were due to: Serratia liquefaciens (from RBC) and Escherichia coli and Bacillus cereus (from platelets). The single fatal bacterial infection was due to Staphylococcus aureus in a unit of platelets.13

The French Hemovigilance Study was conducted from 1994 to 1999 in an effort to collect and analyze information from transfusion related incidents. In this program, all transfusion-related incidents regardless of severity were required to be reported to The French Blood Agency (now The French Agency of Medical Safety of Health Products). Of the 730 incidents suspected to be caused by bacteria, 185 were confirmed as bacterially-related with 89 from platelet components and 113 from RBC. There were 18 fatalities. The overall incidence of bacterial contamination was 12.6 per million components. Fifty-eight percent of the bacteria isolated in RBC were gram-positive cocci - primarily Staphylococcus sp and Streptococcus sp., with gram-negative bacilli identified in 32% of the cases, and the remaining 10% of cases were due to other types of bacteria. In platelet concentrates, gram-negative organisms were isolated in 36% of the units; gram-positive cocci in 42%, and other bacteria in 22%.14

The Bacthem Study was conducted within the French Hemovigilance Network from 1996 to 1998. This matched case control study assessed risk factors associated with transfusion associated bacterial contamination. During this time 41 cases of transfusion associated bacterial contamination met the criteria for inclusion in the study. Of these, 25 were due to contamination in red cell components and 16 due to contamination in platelets. There were six fatalities - four due to RBC contamination and two due to apheresis platelet contamination. Gram-negative bacilli were responsible for 52% of contaminants in red cell components versus 37% of contaminants in platelets. Based on the number of units transfused, the risk of contamination was three times higher with platelets than with RBC transfusions and increased to 12 times when platelets were pooled. In addition, the risk of contamination increased if the platelet concentrates had been stored longer than one day or RBC longer than eight days.11

One of the conclusions from this study was that there is a strong association among type of component, age of component at transfusion, and the risk of transfusion-associated bacterial contamination.

In the U.S. the BaCon study was undertaken to assess the rate of adverse reactions due to bacterial contamination of blood products, to identify the organisms associated with the reactions, and to identify the risk factors for contamination. It was conducted from 1998 through 2000 as a voluntary joint effort among the American Red Cross, Department of Defense, and the American Association of Blood Banks.12 During the first two years there were a total of 103 reports of reactions suspected to be due to bacterial contamination. Of these, 34 reports met the criteria for the study (Table 1). There were nine fatalities reported.12 The most common gram-positive organism isolated was Staphylococcus epidermidis (eight isolates), followed by S. aureus (four isolates). Of the gram-negative organisms, five isolates were Escherichia coli and five Serratia sp. (three S. marcescens, two S. liquefaciens). Patients who received units contaminated with gram-negative organisms were more likely to have severe reactions than those who received units contaminated with gram-positive organisms. Although some authors had previously shown that bacterial contamination and septic reactions occurred up to five times more frequently with pooled platelet concentrates than with single donor platelets, in the BaCon study there were four fatalities due to single donor platelets and two due to pooled platelets.12,18,44

Methods to prevent or detect bacterial contamination

With the ultimate aim of preventing serious reactions due to bacterial sepsis, the American Association of Blood Banks (AABB) has added a new standard to the proposed 22nd edition of Standards for Blood Banks and Transfusion Services. This standard requires blood banks or transfusion services to have methods that limit and detect bacterial contamination in all platelet components. Facilities are expected to implement this by March 1, 2004.46

Over the years, a variety of methods has been suggested to limit and/or detect bacterial contamination. These include changes in aseptic technique for phlebotomy, diversion of the initial aliquot of blood collected, leukoreduction of blood, use of single donor apheresis platelets, use of gram stain or automated blood culture to detect bacterial growth, and...
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pathogen inactivation. Although RBC and platelets are the most commonly contaminated components, fresh frozen plasma and cryoprecipitate may also be contaminated. Because of the relatively infrequent reports of bacterial-contaminated plasma products, this section will focus on methods to detect and/or prevent bacterial contamination of RBC and platelets. The detection of bacteria in units post-collection is necessary, but the improvement of methods to decrease contamination during collection should also be addressed. The entry of normal skin flora with the initial skin plug or from a skin flap formed by the needle bore during donation are two methods theorized to induce contamination. Comparative studies of donor skin disinfection methods have shown that a combination of isopropyl alcohol and povidine iodine would give the greatest reduction in bacterial skin counts (greater than 99% reduction) or platelet contamination rates.47-49

A second prevention approach is to decrease the number of skin bacteria that reach the blood unit. Model systems were developed to compare bacterial concentration in the first few milliliters of blood into the blood bag versus the concentration in the latter part of the flow. Results from several studies showed that the initial 10 to 15 mL draw had the highest bacterial contamination and that by diverting this from the unit, there was a significant drop in percentage of contaminated units.50-52 deKorte noted that, after diversion of this initial aliquot, the prevalence rate of bacteria in units decreased to 0.21% compared to a 0.35% rate in a previous study in which blood was collected using a standard collection method. A significant drop in contamination by staphylococcal species (from 0.14% to 0.03%) was seen after diversion.38 Diverted blood is collected in an attached pouch and can be used for serologic testing or in some cases sampled for bacterial growth. Within the last six months, the FDA has given approval to market a diversion pouch on blood bags in the United States.

Leukoreduction has been instituted in many countries to decrease the risk of HLA immunization, cytomegalovirus transmission, and the incidence of febrile, non-hemolytic transfusion reactions. It has also been suggested that use of prestorage leukoreduction will decrease the growth of Y. enterocolitica in blood units because white cells containing phagocytized bacteria will be removed before cells disintegrate and release the bacteria. A number of studies have shown decreases in rates of Y. enterocolitica contamination using prestorage leukoreduction.11,20,53 The predominance of organisms other than Y. enterocolitica in contaminated RBC in the Bacthem study may be due to use of leukoreduced units in the study.11 Results from a study by Holden in which 19 strains of coagulase-negative Staphylococcus were inoculated into whole blood prior to leukoreduction and component preparation showed that leukoreduction reduced, but did not eliminate bacterial contamination.37 The impact of leukoreduction was also studied as part of the Hemovigilance project. Prevalence of contamination was compared in 18-month periods prior to, and just after, leukoreduction was instituted. There was a significant decrease in both the percentage of contaminated units (3.8% pre-leukoreduction to 1.7% post-leukoreduction) and in the number of septic reactions (71 to 24).38

The use of single donor apheresis platelets instead of pooled platelets has been suggested as another way to decrease risk of septic events. Data from a 12-year study in which platelet units implicated in a febrile, non-hemolytic reaction were cultured indicated that as the percentage of single donor platelet transfusions increased, the rate of septic platelet reactions decreased.44 Several studies using gram stain and bacterial culture demonstrated that bacterial contamination was higher in pooled platelet concentrate than in single donor apheresis platelets, while one study showed higher rates of contamination in apheresis units.15,20,54

Some initial platelet surveillance programs used gram's stain and bacterial culture at the time of platelet transfusion and compared results with storage age of the platelets. The contamination rate was lowest in units stored less than four days. The authors concluded that risk of bacterial contamination was related to duration of storage.18 A major limitation is that the gram's stain is usually not positive until bacteria reach levels of $10^4$ to $10^5$ CFU/mL, which is often on the fourth or fifth day of storage. Another microscopy approach involves the use of a fluorescent nucleic stain applied to the platelet sample with enumeration of the bacteria performed using an epifluorescent microscope. This method is able to detect bacteria at concentrations similar to that of the gram's stain.55 Screening methods using visual changes or measurement of biochemical parameters to detect bacterial contamination have also been studied.56-60 One screening method suggested for detecting possible bacterial contamination in RBC was visual observation of the unit.57,59 RBC units with large numbers of bacteria often show hemolysis or darkening of plasma as compared to blood in the attached segments. Brecher inoculated RBC with either Y. enterocolitica or S. liquefaciens and observed for the presence of hemolysis. Aliquots were sampled for glucose concentration. As bacterial growth increased, the presence of hemolysis increased and the glucose concentration decreased.57
Although bacterial contamination of RBC is usually from the donor, external causes cannot be excluded. Case reports have linked contamination of RBC by Burkholderia cepacia (an environmental organism) to contaminated chlorhexidine solution used in disinfection of donor arm prior to phlebotomy as well as the presence of S. marcescens as a contaminated lot of blood bags.15,16

Platelet concentrates are normally cloudy, therefore visual evidence of bacterial growth is not a useful screening method. Surrogate tests such as decreased glucose concentration (less than 250 mg/dL) or a pH less than 7.0 in platelet concentrate as well as changes in platelet swirling patterns have been suggested as results indicative of bacterial contamination.22,60

Several studies using a number of different bacterial strains associated with platelet contamination were conducted to evaluate changes in platelet plasma glucose and pH using qualitative and semi-quantitative dipstick techniques.15,51,60 The swirling pattern of normal discoid platelets, which is disrupted by platelet shape alterations due to plasma pH changes from bacteria, was visually evaluated. Bacterial concentration reached 10⁷ to 10⁸ CFU/mL before any of these methods detected changes. Results indicated that these methods were less sensitive than microscopic examination of a Gram's stained smear.15,51,60

A number of studies have evaluated the use of an automated blood culture system that detects increased CO₂ as an indicator of bacterial contamination in platelet concentrates.63-67 A variety of organisms (normal flora and pathogens), inoculum sizes, and spiking techniques were used in these studies. Some studies used a special blood collection system containing an integral bag used for sampling that allowed sequential sampling.63,65 Brecher inoculated apheresis units with either 10 CFU/mL or 100 CFU/mL of one of 15 strains of bacteria. All but one of the organisms (P. acnes) were detected within 10 to 26 hours.63 Results from a study comparing growth of normal skin flora and pathogens at inoculum levels of 10 to 100 CFU/mL in both apheresis platelets and random donor platelets demonstrated that bacteria were detected in 98% of the platelet units within 24 hours and in all units within 48 hours, regardless of initial inoculum size.66

A novel approach to detect contaminated platelets involves incubating a sample with a fluorescent-labeled vancomycin probe and then examining it by microvolume fluorometry. The method detected contamination at 10⁵ CFU/mL.68

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Based on results of studies, the most rapid effective and sensitive method (to one CFU/mL) to detect bacterially contaminated platelets is use of an automated blood culture system. Major limitations to this method are that platelets must be stored at least one day for bacterial growth to begin before samples can be inoculated into the blood culture medium and growth must be monitored for another 24 hours.

Within the last year, the FDA has given approval for one company to market the newest system to detect bacterial contamination in platelets—the Pall bacterial detection system (BDS® System). The method uses O₂ levels as a surrogate marker of bacterial contamination. A small aliquot of platelet concentrate is passed over an in-line filter that allows bacteria to move into a small pouch containing growth media but keeps cells back. The pouch is incubated at 35 °C for 24 hours and then tested at room temperature for oxygen concentration. Decreased levels are indicative of bacterial contamination and results are reported as ‘pass’ or ‘fail’.

INACTIVATION OF BACTERIA

Ideally, instead of having to detect and discard units that are contaminated, any organism present could simply be inactivated. Methods using photochemical treatment focus on inactivating bacteria, viruses, and parasites that are present in the component. Many of the compounds described are targeted for platelet concentrates since these components carry the highest rate of bacterial contamination but there are methods reported for RBC.69-73 The most common procedure for platelets involves the combination of a psoralen compound which intercalates with DNA and RNA in the organism, and ultraviolet A (UVA) light. The combination of compound and light causes crosslinking of molecules, which in turn, inhibits replication and transcription. The process does not appear to affect the hemostatic function of platelets.70 Other similar procedures use thionine and short wavelength ultraviolet light.69 The psoralen methods have also been used to successfully inactivate the intracellular bacteria Orientia tsutsugamushi (the etiologic agent of scrub typhus) in platelet concentrates.74

A FINAL LOOK—THE OLDEST AND THE NEWEST

Syphilis is perhaps the oldest known transfusion-transmitted disease and when blood was transfused directly from donor to recipient it was the most common transfusion-transmitted infectious disease. But now, due to improved donor screening tests, medical history questions to identify high-risk donors, refrigeration of units, and the decrease in syphi-
lis in the general population, cases of transmission are rare. In fact, the last reported case was in 1969. Despite these facts and the lack of consensus about need to retain the test, there is concern about the remote possibility of survival of the organism in platelet concentrate, so that syphilis screening of donors continues to be required.76,77

The May 2003 issue of Transfusion published a study by Hedin that investigated the transfusion-transmission potential of Chlamydia pneumoniae. This intracellular bacterium has the potential to be transmitted in white blood cells via transfusion. Although results of this initial study using leukodepleted blood did not yield serological evidence of transfusion-transmission, the authors point out that further investigation should be conducted before adding it to or deleting it from the ever-growing list of transfusion-transmitted organisms.

CONCLUSION

In summary, bacterial contamination remains a major cause of sepsis in the transfused patient. Psychrophilic such as Y. enterocolitica predominate as causative organisms in RBC components and normal flora in platelet components. As a result of data from national studies on prevalence and studies on optimizing detection methods, new AABB standards for prevention and detection of bacterial contamination have been developed. An AABB Association Bulletin from May 2003 has summarized procedures that can be used to help facilities implement the new standards dealing with detection of bacterial contamination.79 The methods include those discussed in this article including improved disinfection, use of diversion techniques, and various methods to detect contamination. Implementation of these methods provide the transfusion community with another challenge in the on-going effort to make a transfusion as safe as possible for the patient.

REFERENCES

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Please indicate your selection of the best article for 2003 from the four eligible issues of CLS, volume 16, issues 1 through 4. The nominated article can be from any section of the journal.

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Transfusion-Transmitted Parasites

LINDA A SMITH, MICHELLE S WRIGHT-KANUTH

ABBREVIATIONS: CDC = Centers for Disease Control; ELISA = enzyme linked immunosorbent assay; HGE = human granulocytic ehrlichiosis; HME = human monocytic ehrlichiosis; HRP-2 = histidine-rich protein 2; IFA = indirect fluorescent antibody; PCR = polymerase chain reaction; pLDH = lactate dehydrogenase; RBC = red blood cells.

INDEX TERMS: parasites; transfusions.

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The incidence of transfusion-transmitted parasitic infections is even lower when compared to that of bacterial and viral contamination; but nonetheless, these organisms may pose a significant risk of illness, especially in immunocompromised recipients. Unlike bacterial contamination, which can occur at multiple points during the collection and transfusion process, transfusion-transmitted parasitic diseases originate from the donor. The most common parasitic organisms implicated in transfusion-transmitted infections are: Plasmodium sp., the causative agent of malaria; Trypanosoma cruzi, the agent responsible for Chagas' disease; and Babesia microti, the etiologic agent of babesiosis. These organisms are gaining prominence due to global travel and increased exposure to habitats where insect vectors reside. Population migration from endemic countries to the U.S. also contributes to the recent increases in parasite transmission. In this article we will discuss the organisms and their prevalence as well as detection methods as they relate to transfusion transmission.

Despite the fact that organisms such as Ehrlichia are bacteria, they will be discussed in this section because they are obligate intracellular organisms that can be transmitted via cells present in a transfusion. They are not normal flora or environmental contaminants – they are primarily insect-transmitted, as are the parasites discussed. These organisms, which have the potential to be transfusion-transmitted, but have limited evidence documenting transmission via blood components, merit discussion.

MALARIA

Transmission of malaria via blood transfusion is relatively uncommon in the U.S. Over the past 30 years the incidence has decreased and is now estimated at less than one case/million units.

In most cases, the implicated donor had visited an endemic area or had emigrated from such a region. There were 1,402 cases of malaria reported in the U.S. in 2000. Two of the cases were congenital and two were blood related.

A comprehensive review of transfusion-transmitted malaria identified 93 cases that had been reported to the Centers for Disease Control (CDC) between 1963 and 1999. Of these patients, 11% (10/93) died. Sixty-five percent of all cases came from the following areas in order of number of cases—New York City (NYC), Texas, California, New York State (excluding NYC), Pennsylvania, and Florida. During this period, the total number of cases caused by each species was: P. vivax – 25, P. malaria – 25, P. ovale – 5, and P. falciparum – 33, with the remainder either reported as a mixed infection or as Plasmodium sp. Table 1 gives the distribution of cases by decade. The percentage of cases due to P. falciparum increased from 47% during 1963–69 to 71% in the period 1990–99. This is probably attributable to increased immigration from areas such as sub-Sahara Africa where P. falciparum is the primary species and where drug resistant strains have developed. Most cases were associated with trans-
fusion of erythrocytes, but approximately 6% were linked to platelet transfusion. This is most likely due to the presence of residual red blood cells (RBC) in the platelet concentrates.

At the present time, there are no approved methods to screen blood donors for malaria in either the U.S. or Canada. Medical and travel history elicited through the questions asked of the donor prior to donation is the only way to determine if the donor poses a risk of transmitting malaria. In one study it was determined that 62% of the accepted donors who were implicated in transfusion-transmitted malaria should have been excluded based on the donor guidelines in place during the time periods studied. In the remainder of the cases, however, the donor had returned from travel to endemic areas or had been in the U.S. longer than the minimum deferral period.

All three cases of transfusion-transmitted malaria in Canada during 1994-99 were due to P. falciparum. Two of the cases were due to contaminated RBC and one due to contaminated platelets. The infections were detected in recipients by a positive blood smear. All implicated donors had met screening guidelines for donation, yet two of the three donors had a positive smear. As a result of these cases, Canadian guidelines were changed in 1995 to permanently defer a person who has a past history of diagnosis of and/or treatment for malaria. Conversely, a review of three U.S. cases of transfusion-transmitted malaria during 1996-1998 showed that donors had been accepted for donation based on their responses to questions, but in actuality they did not meet guidelines in place at the time of donation.

The problem of individuals who meet donor guidelines for travel, yet become implicated in transfusion transmitted malaria may be due to low levels of ongoing parasitemia. Persons in highly malarious areas may have persistent asymptomatic parasitemia due to an acquired immunity or inadequate treatment. In most instances, parasitemia due to P. falciparum is eliminated in two years, while P. vivax and P. ovale parasitemia may persist three to five years. P. malariae has recurred after 40 years. In one report there was a 15-year gap between exposure to malaria and a blood donation that transmitted P. falciparum.

Aside from donor screening, other options to identify infected donors include use of tests primarily designed to detect parasites in symptomatic individuals or antibody screening tests. Tests for detection of parasites include:

- Thick/thin blood smears, fluorescent staining techniques, tests for circulating malarial antigen, or polymerase chain reaction (PCR) for detecting malarial DNA.

Examination of thick blood smears is not cost-effective for screening large numbers of donors, nor is it sensitive enough (limit of detection is approximately ten organisms/µL) to detect low levels of parasitemia that might exist in donors. Less than 50% of implicated donors in studies had positive smears, which is probably related to low levels of circulating parasites. Fluorescent stains such as acridine orange that stain nucleic acids can also be used for examination of a thick film for parasites or in systems that employ capillary tubes filled with blood (QBC™). Commercial systems, however require expensive equipment and do not allow for retention of the specimen. With these systems, the species identification must be done using a thin blood smear.

In recent years, there has been increased use of dipstick tests for rapid screening/diagnosis of acute malaria in rural endemic areas. These tests, which use monoclonal antibody fixed to nitrocellulose strips detect circulating P. falciparum histidine-rich protein 2 (HRP-2) antigen or Plasmodium lactate dehydrogenase (pLDH). The level of detection required in acute cases is approximately 500 parasites/µL, which may be greater than the level of circulating parasites in an asymptomatic blood donor.

On the other hand, serologic tests identify antibody positive individuals, but do not indicate parasitemia because antibody levels can remain elevated up to ten years after infection. In general, when used in a donor population with a low prevalence of malaria, antibody

---

**Table 1. Number of cases of transfusion transmitted malaria 1963-1999**

<table>
<thead>
<tr>
<th>Years</th>
<th>P. vivax</th>
<th>P. ovale</th>
<th>P. malariae</th>
<th>P. falciparum</th>
<th>Other*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963-69</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>1970-79</td>
<td>14</td>
<td>1</td>
<td>8</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>1980-89</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1990-99</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>—</td>
</tr>
</tbody>
</table>

*Mixed infections or Plasmodium sp.*
tests have poor positive predictive value. Laboratories in France, however, use an indirect fluorescent antibody (IFA) method to detect antibodies to Plasmodium sp. in at-risk donors. There has not been a reported case of transfusion-transmitted malaria since the test was instituted in 1994.7

One study evaluating an enzyme linked immunosorbent assay (ELISA) test that used P. falciparum antigen indicated that the test may be sensitive and specific enough to screen for antibodies in at-risk donors whose medical history indicated they might have been exposed to malaria.11

Silvie used a combination of a P. falciparum HRP-2 antigen test and an enzyme immunoassay (EIA) antibody test to test plasma specimens from patients with confirmed P. falciparum infection.7 Results in patients with confirmed malarial infection indicated that the combination of tests detected more positives than either test alone. Again, there is concern whether even this combination can detect the very low levels of parasitemia often seen in donors; especially since small amounts of blood are used.7

PCR methods to detect plasmodium DNA or RNA may be the most sensitive (one parasite/50 µL) and specific but are technically demanding and the most expensive. One study, however, has indicated even this method may not be able to detect organisms below the level of 10 parasites/10µL blood.7

As with bacterial contamination, the best approach may be to find agents that will inactivate the parasite in donor blood. A report describing a recent pathogen inactivation system for bacteria (INTERCEPT™) that uses a psoralen-type compound and ultraviolet light showed that P. falciparum is susceptible to inactivation with this system.12

CHAGAS’ DISEASE (AMERICAN Trypanosomiasis)

Chagas’ disease, caused by the hemoflagellate, Trypanosoma cruzi is endemic in Central and South America and parts of Mexico. It is transmitted by an insect vector commonly called the ‘kissing bug’ (a member of the Reduviidae family). In order to understand why there is potential risk of transfusion transmission, the life cycle of the organism needs to be reviewed. The disease is initially acquired when the infective trypomastigote stage is deposited on human skin in the insect’s feces after it takes a blood meal. The organism enters the human circulation through a break in the skin. The acute stage of the illness is short-lived and characterized by fever, anorexia, hepatosplenomegaly, and circulation of the trypomastigote form in blood. About 10% to 30% of those infected will develop chronic trypanosomiasis with intracellular invasion by the organism.13 This intracellular amastigote stage is responsible for the chronic form of the disease which is characterized by neurological disorders, progressive damage to heart muscle – resulting in cardiomyopathy, or damage to the digestive system – resulting in megacolon or megaeosophagus. During the chronic stage infective trypomastigotes circulate in low numbers in the individual’s blood and make the blood potentially capable of transmitting the disease by transfusion.

In endemic areas the seroprevalence of the disease varies from less than 1% to 62% (depending on the country) with estimates of 16 to 18 million persons infected overall.14-17 Blood donors in endemic areas are commonly tested for antibodies before donation and the risk of acquiring transfusion-transmitted Chagas’ disease from seropositive donors in endemic area ranges from 12% to 48%.13,15,18

| Table 2. Sample donor questions related to risk of parasitic infection |
|---------------------------|-----------------------------|
| **Question**              | **Action**                  |
| Have you ever had:        | Follow up questions to determine how long ago and treatment. |
| malaria?                  | Indefinite deferral          |
| Chagas’ disease?          | Indefinite deferral          |
| babesiosis?                |                             |
| In the past three years have you: |                             |
| traveled outside the U.S.?| Follow up questions to assess risk of exposure to malaria or Chagas': |
| country(ies) visited, length of time, rural or urban area | country(ies) visited, length of time, rural or urban area |
| resided in another country?| return date to U.S.          |
| Have you ever had chest pain, heart disease or lung disease? | May be indicative of symptoms of chronic Chagas’ disease |

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FOCUS: TRANSFUSION RISKS
Transfusion transmission is the second most common method of acquiring the disease, followed by transplant-transmitted and finally transplacental (congenital). There have been four reported cases of transfusion-transmitted Chagas' disease in the U.S. and two in Canada, with the majority due to T. cruzi contaminated platelets. The recipients were all immunocompromised and all but one of the donors was from a country endemic for T. cruzi. In one case the donor, who had emigrated to the U.S. 33 years ago, demonstrated circulating trypomastigotes.

Transmission by solid organ transplantation (especially renal transplant) is also common in Latin American countries and has now been documented in the U.S. Three individuals, all of whom had received organs from same donor, developed Chagas' disease including the presence of circulating trypomastigotes.

Despite the few documented cases of transfusion-transmitted T. cruzi infection, there is concern about the safety of the U.S. blood supply because of increased immigration from endemic areas. It is estimated that 25,000 to 100,000 Latin American immigrants in the U.S. may be infected with T. cruzi. In addition, trypomastigotes have been shown to remain viable in stored whole blood for seven days, in platelets for four days, and in RBCs for two days with PCR testing for T. cruzi DNA remaining positive throughout the storage of the units.

Several studies involving blood donors or recipients of blood donations have been carried out to determine risk status and/or seroprevalence in the U.S. In one study, donors in the American Red Cross Southwest region were tested for antibody to T. cruzi using a screening test and repeatedly reactive results were confirmed using recombinant immunoprecipitation assays. There was a confirmed positive rate of 1 in 33,000 (0.003%).

Leiby and colleagues tested donors in Los Angeles and Miami from 1994–98. To establish risk status, donors were first asked a question about residence in, or travel to, a country where Chagas' disease is endemic. Testing of plasma samples from those who responded 'yes' gave an overall confirmed seroprevalence of 0.14%. The seroprevalence of Miami donors stayed relatively constant – 0.09% - during the study period. In contrast, seroprevalence of Los Angeles donors increased from 0.15% to 0.19% during the last two years. This approximates to 1 in 7500 donors who were seropositive in Los Angeles, and 1 in 9000 donors in Miami.

Look back studies of donors who tested positive showed that no recipient of blood products from these donors became seropositive for antibodies to T. cruzi. Another large look back study involving more than 11,000 cardiac surgery patients who received blood showed that only six recipients had antibody to T. cruzi present and all had acquired the infection prior to transfusion. Despite these figures, the frequency of transfusion-transmitted T. cruzi is most likely underestimated due to the mild acute symptoms, long interval until chronic symptoms develop, and lack of testing.

Acute infection is usually diagnosed by observation of the trypomastigotes on a Wright-stained blood smear. However, in the chronic stage the circulating level of trypomastigotes is too low to be detected and therefore seropositivity is used as evidence of infection. Serologic tests using ELISA methodology are sensitive and specific in detecting parasitemia when seroprevalence of the organism is relatively high but they cannot readily distinguish between acute and chronic infection. In non-endemic areas such as the U.S., the positive predictive value of the test is poor.

Another problem with current serologic tests is that the antigens used are derived from whole organisms and some antigens may be shared with other parasites such as Leishmania sp. This yields cross-reactions and false positive results, which may be of more concern in areas such as South and Central America where leishmaniasis is also endemic. A serologic test using four recombinant T. cruzi antigens was evaluated and showed greater than 99% sensitivity, greater than 98% specificity and that it could be used in blood donor screening.

In endemic countries gentian violet (crystal violet) is used to inactivate the organisms. Gentian violet with or without ascorbic acid is added to the blood unit. The reaction of the compound when exposed to light produces a superoxide ion, which further releases products that will kill the protozoan. Use of this product does not appear to affect metabolism or preservation of red cells.

Currently in the U.S., aside from donor questions that may defer at-risk donors, there is no way to identify at-risk donors or prevent transmission. There is no policy requiring serologic testing of donors for antibodies to T. cruzi. Although there are two ELA kits available in the U.S., neither is licensed for screening blood donors. PCR, which is not available for general testing, may someday be used to detect circulating antigens in at-risk donors.
BABESIOSIS

Babesiosis, an arthropod transmitted disease, is endemic in the Northeast (New York, Connecticut, Massachusetts) and parts of the upper Midwest including Minnesota and Wisconsin. After malaria, it is the most common transfusion-transmitted parasitic disease. It is an intraerythrocytic parasite that like malaria, can be transmitted not only by RBC transfusion but also by the few RBC present in a unit of platelets. The organisms usually associated with human infection, Babesia microti and B. divergens, are transmitted by the bite of a tick (Ixodes sp.). In the last five years, WA1-type and MO1-type Babesia sp. were also identified as etiologic agents of babesiosis and the WA1-type has been linked to transfusion-transmitted babesiosis. Although over 30 cases of transfusion-transmitted babesiosis have been reported in the U.S. since 1979, the overall incidence is less than one per one million units of blood. However, in endemic areas the risk is higher—up to six cases/million units. Reports of seroprevalence from areas such as New York and Connecticut vary widely from 0.6% to greater than 6.9% of donors positive for antibodies in endemic areas to less than 2% positive in non-endemic areas.

Most tick-caused infections are asymptomatic or exhibit non-specific symptoms such as fever, fatigue, chills, and anorexia. In elderly, asplenic, or immunocompromised patients a severe malaria-like illness with hemoglobinuria, hemolytic anemia, and renal failure can occur. Asymptomatic parasitemia in untreated patients persists for at least a year. B. microti organisms can survive at 4 °C and at 25 °C. One study demonstrated that organisms in blood stored at 4 °C were viable at day 17; those in blood held at 25 °C were viable for three days. Results from a study that monitored Babesia-infected subjects every three months for up to 27 months demonstrated organisms in the circulation (blood smear) for approximately a week but PCR assays for circulating babesial DNA were positive for 82 days. In a cluster of incidents in New York, five of eight patients who received infected blood became infected. Two recipients, each a chronically transfused patient, were infected by single donor and developed smear positive evidence of infection. The third case in the cluster involved a single donor who infected six patients from the same unit, four of the infected were neonates who received aliquots of blood from the unit. A blood smear from the unit demonstrated a single infected cell. Transfusion-transmitted cases have also been reported in transplant patients who receive large quantities of blood products. Just recently, a case of transfusion-transmitted babesiosis was identified in Canada.

Complicated donor had a positive blood smear and a 1:1024 antibody titer. A case from the upper Midwest involved a donor who infected multiple individuals over a six-month period through blood donations. Four of seven individuals who received components from this donor became infected. A review of donor’s medical history showed that he had most likely been parasitemic for at least ten months.

As with malaria and Chagas’ disease, there are no approved serological tests for donor screening and donor questions may not always elicit correct history since the infection is often asymptomatic. Current diagnostic tests for babesiosis are not suited to large-scale donor screening. Examination of peripheral blood smears, indirect fluorescent antibody tests, PCR for detection of B. microti specific targets, or inoculation of animals are slow, costly, and/or labor-intensive methods. Efforts are underway to develop ELISA tests for detection of babesial antibodies that might be suitable for donor screening. Methods for pathogen inactivation to prevent transmission of babesiosis have been reported. At the present time, however, any donor with a history of babesiosis is indefinitely deferred because of the possibility of persistent parasitemia.

EHRLICHIOSIS

There are two human ehrlichioses – human monocytic ehrlichiosis (HME) caused by Ehrlichia chaffeensis and human granulocytic ehrlichiosis (HGE) caused by organisms that resemble E. equi and Anaplasma phagocytophila (formerly E. phagocytophila). The human ehrlichioses are characterized by clinical symptoms that are similar to Lyme disease and Rocky Mountain spotted fever. One distinguishing factor, however, is the presence of intracytoplasmic inclusions in white blood cells. These morulae, as they are called, are actually intracellular bacterial microcolonies that can be seen on a Wright’s stained blood smear.

Although there are several cases of HGE and HME linked to transplant transmission, only one case of HGE has been linked to transfusion transmission. However, E. chaffeensis can remain viable in blood for up to 11 days and A. phagocytophila for up to 18 days. A seroprevalence study of antibodies using an IFA technique to detect antibodies to A. phagocytophila in random blood donors was conducted in Wisconsin and Connecticut. Seropositivity ranged from 0.5% in Wisconsin donors to 3.5% in Connecticut donors. At this time there are no screening questions asked of the blood donor to determine at-risk status for ehrlichiosis.
OTHER POTENTIAL TRANSFUSION-TRANSMITTED DISEASES

Ticks are effective vectors of disease and are responsible for transmission of a number of viral, spirochetal, and rickettsial agents that cause serious diseases. In addition to B. microti, and the etiologic agents of ehrlichiosis, ticks can transmit the causative agents of Lyme disease and the spotted fever group, all of which have the potential to be transfusion-transmitted.

Lyme disease, caused by spirochete Borrelia burgdorferi, can be transmitted concurrently with B. microti or ehrlichiosis by the same insect vector. Individuals who show symptoms of one disease often have positive antibody titers to both organisms. Despite the numerous cases of transfusion-transmitted babesiosis, however, there have been no reported cases of transfusion-transmitted Lyme disease. Although B. burgdorferi can survive in red cells stored at 4 °C up to a month and in platelets stored at room temperature, it appears to circulate in the blood for a relatively short time.

Rocky Mountain Spotted Fever is caused by the obligate intracellular bacillus Rickettsia rickettsii. Despite potential for transfusion transmission, it is not common. There is only a single case of documented transfusion transmission in the late 1970s. A report concerning 377 donors from a National Guard unit who were exposed to ticks and who developed symptoms of tick-borne illness (Rocky Mountain spotted fever or ehrlichiosis) demonstrated that none of the recipients of components from the 12 confirmed cases developed serologic evidence of exposure.

Leishmania donovani, the etiologic agent of visceral leishmaniasis is transmitted by the bite of a sandfly. The organism is an intracellular parasite that is present primarily in cells of the reticuloendothelial tissue and cells of the mononuclear phagocytic system. In endemic areas such as Africa, Asia, and South America there is a relatively high seroprevalence (24% to 43%) of antibodies to the organism. After the 1980s Gulf War, returning military personnel were deferred from donating blood due to fears of possible transfusion-transmission of the organism, as well as from a viscerotropic L. tropica. Studies in animals have shown that it can be transfusion-transmitted, but documented cases are rare. Because the organism is present in circulating phagocytic cells, one study compared the presence of parasite DNA in blood pre- and post-leukodepletion. Results demonstrated that after leukodepletion, there was no parasite DNA present in the blood.

In summary, the transmission of parasitic organisms through transfusion is relatively rare. Of the three major transfusion-transmitted diseases, babesiosis and Chagas' disease pose the greatest threat to donors in the U.S. In both cases, this is due to the increased number of potentially infective donors. There are no serologic tests available to screen donors for any of these organisms and the focus for prevention remains on adherence to donor screening guidelines that address travel history and previous infection with the etiologic agent. One goal is the development of tests that are able to screen for and identify donors potentially infectious for T. cruzi or B. microti, without causing the deferral of a large number of noninfectious donors or significantly increasing costs. Ideally, methods to inactivate the infectious organism will provide an element of added safety to the blood supply.

REFERENCES

FOCUS: TRANSFUSION RISKS


References continued on page 251.
FOCUS: TRANSFUSION RISKS

Continuing Education Questions

To receive 4.0 contact hours of intermediate level P.A.C.E., credit for the Focus: Transfusion Risks questions, insert your answers in the appropriate spots on the immediately following page; then complete and mail the form as directed.

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

LEARNING OBJECTIVES
Please see page 220 for the learning objectives for this Focus Section.

CONTINUING EDUCATION QUESTIONS

1. NAT testing on donor units may soon be required to detect:
   a. CMV.
   b. HGV.
   c. West Nile virus.
   d. SEN virus.

2. The blood supply in the U.S. is currently tested for which of the following viruses?
   a. HTLV I
   b. CMV
   c. EBV
   d. HAV

3. NAT testing of donor units for HAV has been proposed because:
   a. HAV is ubiquitous in the U.S. population.
   b. transfusion-transmission of HAV is postulated to be possible.
   c. it has been shown that some cases of HAV are transfusion-transmitted.
   d. all non-A, non-B, non-C hepatitis is caused by HAV.

4. The implementation of NAT testing has reduced the incidence of transfusion-transmitted HCV to approximately:
   a. 1 in 614,000.
   b. 1 in 1,600,000.
   c. 1 in 103,000.
   d. 1 in 1 billion.

5. S-D treatment of FFP will NOT inactivate:
   a. HBV.
   b. HCV.
   c. WNV.
   d. Parvovirus B19.

6. Psoralens will intercalate with nucleic acids when activated by:
   a. ultraviolet A.
   b. ultraviolet B.
   c. methylene blue.
   d. caprylate.

7. The detergent used in S-D treatment of FFP:
   a. disrupts lipid envelopes.
   b. requires white light to function.
   c. forms a photoadduct with nucleic acids.
   d. requires pre-filtration to function.

8. A 14-day-old unit of donor red blood cells appears hemolyzed when selected from the blood bank refrigerator for crossmatching. If hemolysis is due to bacterial contamination, what organism is most likely involved?
   a. Staphylococcus epidermidis
   b. Bacillus cereus
   c. Babesia microti
   d. Yersinia enterocolitica

9. The most common group of organisms associated with platelet contamination is:
   a. normal skin flora.
   b. environmental organisms.
   c. gram-negative rods.
   d. microaerophilic organisms.
FOCUS: TRANSFUSION RISKS

10. The most sensitive detection method available for bacterial contamination in platelets is:
   a. Gram's stain.
   b. automated blood culture system.
   c. fluorescent microscopy.
   d. measurement of pH and glucose.

11. The risk of bacterial contamination is greatest with which of the following components?
   a. Red blood cells
   b. Apheresis platelets
   c. Pooled platelets
   d. Plasma products

12. The underlying cause of death in patients who receive red cells contaminated with Yersinia enterocolitica or Ser- ratia liquefaciens is:
   a. septic shock due to endotoxin.
   b. DIC due to intravascular hemolysis.
   c. thrombosis induced by endotoxin.
   d. kidney failure due to free hemoglobin.

13. The major factor that encourages growth of bacteria in platelet concentrates is the:
   a. use of leukoreduction techniques.
   b. pH of unit.
   c. limited amount of plasma present.
   d. storage temperature.

14. The minimum level of bacterial contamination detected by microscopy with Gram's stain is:
   a. $10^2 - 10^3$ CFU/mL.
   b. $10^4 - 10^5$ CFU/mL.
   c. $10^7 - 10^8$ CFU/mL.
   d. $>10^9$ CFU/mL.

15. The major method of bacterial inactivation used for platelets is:
   a. irradiation.
   b. psoralen compounds.
   c. gentian violet with ultraviolet light.
   d. solvent-detergent treatment.

16. The major transfusion-transmitted parasite that is endemic in the U.S. is:
   a. Plasmodium falciparum.
   b. Trypanosoma cruzi.
   c. Babesia microti.
   d. Ehrlichia chaffeensis.

17. Which of the following organisms is of concern in donors from Central and South America?
   a. Leishmania donovani
   b. Ehrlichia chaffeensis
   c. Trypanosoma cruzi
   d. Plasmodium vivax

18. Prolonged asymptomatic parasitemia is responsible for the transfusion transmission of all of the following EXCEPT:
   a. babesiosis.
   b. malaria.
   c. Chagas disease.
   d. ehrlichiosis.

19. The combination of gentian violet, ascorbic acid, and exposure to light will inactivate which of the following organisms?
   a. Trypanosoma cruzi
   b. Plasmodium sp.
   c. Babesia microti
   d. Anaplasma phagocytophilia

20. Donor screening questions address risks for all of the following diseases EXCEPT:
   a. malaria.
   b. ehrlichiosis.
   c. Chagas' disease.
   d. babesiosis.

21. The greatest percentage of transfusion-transmitted cases of malaria in the U.S. is due to which organism?
   a. P. falciparum
   b. P. malariae
   c. P. ovale
   d. P. vivax
Continuing Education Registration Form

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

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Answers

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

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

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

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

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

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

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

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

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

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

Mary Jane Gore

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

FDA APPROVALS

The U.S. Food and Drug Administration has approved the first rapid assay screening test that physicians can use in their offices to help diagnose patients with bladder cancer. The FDA approved Matritech’s NMP22® BladderChek™ test, which detects a bladder cancer nuclear matrix protein in urine of patients. Contact (617) 928-0820, ext. 248, or visit www.matritech.com.

NEW PRODUCTS

Tecan US has announced a strategic alliance with GE Medical Systems for Tecan’s front-end Genesis FE500 Workcell. Together, these companies plan to enhance clinical diagnostics lab output using Tecan’s state-of-the-art robotic equipment to enhance sample flow, coupled by world-class customer service by GE Medical Systems. As a modular, compact front-end automation workcell, the Genesis FE500 combines all pre-analytical functions including pre-sorting, centrifugation, volume check and clot detection, decapping, secondary tube labeling, aliquotting and destination sorting into analyzer racks from a variety of manufacturers. Contact GE Medical Systems Britt Zarling, at (262) 544-3453.

Leica Microsystems introduces the new Leica IP C cassette and IP S slide printers for printing permanent, legible information directly onto most standard tissue cassettes and microscope slides used in histology cytology and biomedical research laboratories. Contact Molly Lundberg at (847) 405-7026.

Using a new patent-pending communications technology that bypasses traditional telephones—and their toll charges—a California staffing agency has reduced its search time to fill a job opening from an average of two hours to two minutes. Designers of the technology believe it offers time- and cost-saving efficiencies that are applicable to the personnel industry, and emergency services, education, and other industries. This technology, called One Call On Call, combines a variety of existing communications formats into one system that renders the traditional telephone unnecessary and accelerates connectivity, says W. Denis Nurmela, founder and chief executive officer of the corporation. For more information, contact Mr. Nurmela at (866) 842-5150 or denis@onecalloncall.com.

The French biotech company GeneSystems has invented a very-high-speed technology for microbiological analysis, the GeneDisc Cycler. This technology is based on a principle drawn from the field of molecular biology: real time PCR (polymerase chain reaction) and makes use of a consumable product, the GeneDisc. GeneSystems controls all stages in the development of the GeneDisc Cycler, from design to marketing. For more information, contact Alicia Stanley at (312) 327-5260 or visit www.genesystems.fr.

GeneDisc cycler
TRENDS AND TECHNOLOGY

Thales Optem has introduced the Universal Digital Camera Coupler for connecting almost any digital camera featuring an accessible female thread to a wide range of optical microscopes. This economical and versatile camera coupler features a 37mm (M 37 x 0.75) mounting thread and is compatible with a variety of step-down rings and adapters. This allows maximum versatility for quick and easy integration. Two key functional features stand out: a unique adjustment, which minimizes vignetting across the entire zoom range of the digital camera and the ability to both record the microscope’s maximum image diameter at low zoom while also filling the camera’s format as focal length increases. Contact marketing@thales-optem.com.

Bayer Diagnostics has announced worldwide availability of the Bayer serum HER-2/neu oncprotein test on its leading automated immunoassay platform—the ADVIA Centaur® immunoassay system. The blood test measures circulating levels of the HER-2/neu oncprotein to help oncologists treat patients with metastatic breast cancer. The test was cleared for use on the ADVIA Centaur system by the U.S. Food and Drug Administration earlier this year. Other recent additions to the Centaur system include four fully automated hepatitis B assays and a hepatitis C assay. Contact Amy Samaha at (914) 366-1815.

Roche Diagnostics has four new configurations available for its Integrated Modular ANALYTICS systems. All four combine clinical chemistry and immunassay testing onto one platform to enhance efficiency and productivity. All can be used as a main line or specialty analyzer in a hospital or reference setting. For more about the optimal work type and volume for each configuration, contact Lisa Davis at (800) 428-5074 or visit us.labsystems.roche.com.

INDUSTRY NEWS

To help address the labor crisis in the clinical laboratory industry, Jim Reid-Anderson, chairman, president and CEO of Dade Behring, announced the creation of a $150,000 scholarship fund to be distributed over a three-year period. Fifty $1,000 scholarships are to be distributed each year to individuals seeking associate degrees in medical laboratory science. The first scholarships will be awarded in 2004. The scholarship program will be administered by the Coordinating Council on the Clinical Laboratory Workforce (CCCLW), a group that represents all the clinical professional societies, and four federal agencies, including the FDA, the CDC, and the Veterans Administration. The CCCLW has agreed to develop the criteria for the scholarship application, promote the program on all of their Web sites and in newsletters, and create a judge’s panel to select the winners. Expressing gratitude to Dade Behring, Jim Griffith, the convener of the CCCLW and Chancellor Professor and Chairperson; Department of Medical Laboratory Science at the University of Massachusetts–Dartmouth, said, “It’s a terrific start and we hope it will inspire others in the industry to follow suit”. Contact Pattie O’verstreet-Miller at (847) 267-5426 or James T Griffith PhD CLS (NCA) at (508) 999-8328.

According to Dow Jones Newswires, immunodiagnostics sales could double in five years for Roche Holding AG because of a late July $1.4 billion deal to buy Igen International Inc. (IGEN), said Hino von Prondzynski, chief executive of Roche’s immunodiagnostics business. Prondzynski said, could increase to $900 million in five years from $455 million now, or growth from the current 11% to more than 20% of the
$6.2 billion immunodiagnostics market. The immunodiagnostics division makes tests that can detect thyroid problems, infertility, tumor markers, infectious disease, and cardiac markers. In an interview with Dow Jones, Prondzynski noted that he deals allows Igen to go forward as its own company with applications such as food testing and defense contracts which held no interest to Roche, while Roche gets full access to Igen’s O’rigen technology, for which it had to pay royalties in the past.

Dade Behring, the largest company in the world solely devoted to clinical diagnostics, today released the findings of a benchmark survey on the diagnostic industry’s reputation among U.S. consumers, healthcare professionals, and others. The intent of the summer 2003 survey was to develop further understanding of the expectations of consumers and healthcare and industry professionals, so that the industry could address their needs. Among findings, the survey indicated that half of the consumers interviewed (51 percent) consider that the diagnostics industry has a strong impact on the quality of their health care, and more than half (56%) consider that laboratory testing professionals have a “strong impact” on the quality of health care they receive. More than four out of five (84%) say that early-stage testing to assess one’s risk of developing a disease is important.

Correlactic Systems, Inc., developer of promising new cancer detection tests that use just a single drop of blood from a patient, has entered into an agreement with Advion BioSciences, a leading provider of mass spectrometry services and products, to explore the use of Advion’s NanoMate™ System as a component of Correlactic’s upcoming ovarian cancer clinical trials. Correlactic’s test, called Proteome Pattern Blood TestSM, scans a drop of blood for protein patterns generated by a mass spectrometer. In February 2002, Correlactic Systems earned international recognition when its joint research with the National Cancer Institute (NCI) and the U.S. Food and Drug Administration revealed that the Correlactic test successfully detected all of the ovarian cancer patients in a study of 216 women, including cancers that were still in Stage 1. The goal of the Correlactic-Advion research is to determine whether results similar to those published in February 2002 can be replicated on the NanoMate system and employed in the NCI-sponsored clinical trials.

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

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