Focus: Psychostimulants
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Health Disparities and Public Policy

ISAAC D MONTOYA

Health-related disparities are significant differences in the incidence, prevalence, morbidity, mortality, and burden of disease among specific population groups. Medical research has demonstrated glaring disparities for a wide range of health problems and among different groups. It is important that we improve our understanding of what causes health disparities and work to address them. In doing so there is a tendency to discuss health disparities solely in terms of differences among racial and ethnic groups; however it is a myth that only these groups experience disparities. Unfortunately, health disparities occur in all segments of society.

Medical research working to achieve the goal of alleviating health disparities in the United States is a goal with broad support that adopts the well-established perspective that various forms of discrimination and poverty are the major contributors to unequal health status. One idea that has been put forth is that genetic research plays a significant role in alleviating this national problem, which may overstate the importance of genetics in explaining health disparities. Over reliance on genetics as a factor in explaining health disparities may lead us to miss the factors that we can control, thus reinforcing stereotyping which contributes to disparities in the first place.

Access to care is a primary reason for disparities. For example, disparities due to limited access to coronary artery bypass graft (CABG) surgery are well documented. Evidence shows that even when patients do receive CABG surgery, the poor, people living in rural areas, and racial minorities are more likely to be treated by lower quality providers. In addition, some disparities occur due to the hospital to which patients are admitted and to a lesser degree to being treated by a low-volume surgeon. Efforts to eliminate health disparities should address not only access to care, but also access to high-quality care.

Obesity is a major concern nationally that crosses all socioeconomic groups. Genetics, and lack of access to dietary/nutritional information, healthy foods, and needed exercise all contribute to the problem of weight control. A recent study, funded by the National Institutes of Health, analyzed a sample of Americans’ weight. The results show continuing disparities by sex and between racial/ethnic groups in the prevalence of obesity.

Adults with developmental disabilities experience disparities that are often not as obvious. A recent study found this population to be more likely to lead sedentary lifestyles and seven times as likely to report inadequate emotional support compared to adults without disabilities. Adults with disabilities and developmental disabilities were also more likely to report being in fair or poor health than adults without disabilities. Significant medical care utilization disparities were found for breast and cervical cancer screening as well as for oral healthcare in this group.

Mental health and drug abuse, e.g., nicotine, alcohol, and illicit drug problems, are a classic example of disparities at both the prevention and treatment level. These types of behavioral problems face stigmas that further contribute to the disparity problem. For example, in a national study the prevalence of smoking during pregnancy ranged from 9.0% to 17.4%. Younger (age <25 years) women, white women, American Indian women, non-Hispanic women, women with a high school education or less, and women with low incomes consistently reported the highest rates of smoking. In the same study the prevalence of alcohol use during pregnancy ranged from 3.4% to 9.9%. In seven states, women age >35 years, non-Hispanic women, women with more than a high school education, and women with higher incomes reported the highest prevalence of alcohol use during pregnancy. Although prevalence data cannot be used to identify causes or interventions to improve health outcomes, they do indicate the magnitude of disparities and identify populations that should be targeted for intervention.

We recognize that a number of groups experience inferior medical care and health status, but may not appreciate the seriousness of the problem. Each year the United States spends billions of dollars to perfect the ‘technology’ of healthcare, e.g., development of new drugs, new pieces of equipment, and to modernize delivery systems, thereby saving thousands of lives. Correcting known disparities could prevent five times as many deaths. If policymakers adhered to the goal of optimizing population health, greater priority would go to resolving disparities rather than to developing new technology, but unfortunately reverse priorities prevail.

Isaac D Montoya PhD was the Clinical Laboratory Science Research and Reports Editor, 2001-04.
In today’s climate of focus on the healthcare consumer, much attention is being paid to improving patient safety and quality of care. There is heightened interest in distinguishing healthcare providers who are “good performers”, who provide safe and efficient care from poorer performers whose outcomes may not be as good. Some payers feel that one way to encourage performance improvement is to pay good performers better than other providers.

The Center for Medicare and Medicaid Services (CMS) began a Pay for Performance program for hospitals on a pilot basis about two years ago. Hospitals that participate in the pilot project keep statistics on a number of measures in diagnosis groups common in the Medicare population, such as acute myocardial infarction (AMI), coronary artery bypass grafts (CABG), heart failure, pneumonia, and hip and knee replacement. If hospitals achieve certain levels of compliance with the goals of the measures, they are paid 1% to 2% more than the usual DRG payment.

A few examples of the over thirty pay for performance metrics defined by CMS are:

- AMI: aspirin at arrival
- AMI: thrombolytic within 30 minutes of arrival
- AMI: percutaneous coronary intervention received within 120 minutes of arrival
- CABG: post operative hemorrhage or hematoma
- Heart failure: smoking cessation advice/counseling provided
- Pneumonia: oxygenation assessment within 24 hours
- Pneumonia: blood culture collected prior to first antibiotic assessment
- Hip and knee replacement: prophylactic antibiotic received within one hour prior to surgical incision

On January 31, CMS announced that ten large physician groups across the country would participate in a three-year pilot project of pay for performance for physicians. Physicians will continue to be paid on a fee-for-service basis, but will be eligible for performance payments based on how well they improve patient outcomes and avoid costly complications. The quality measures focus on the many of the same chronic illnesses in the Medicare population as do the hospital measures, including congestive heart failure, coronary artery disease, diabetes, and hypertension, as well as preventive services such as screenings for breast and colorectal cancer, and immunization for flu and pneumonia.

Another proposal related to pay for performance for physicians surfaced in January that would have a very direct impact on the laboratory. During hearings on January 12, the Medicare Payment Advisory Commission (MedPAC) made a recommendation that CMS should require laboratories to report test results to CMS on the claim for payment. From a reading of the transcript of the proceedings of the MedPAC, it is not clear how the data would be used to evaluate physician performance, perhaps by measuring the percentage of abnormal results. In addition to using this strategy to evaluate physician performance for pay for performance, MedPAC discussion focused on the requirement as a way to encourage all providers to use information technology (electronic medical record). The electronic medical record has been a focus of the Bush administration’s health policy.

Laboratorians are likely to be skeptical about the effectiveness of laboratory values alone as a measure of physician effectiveness, taken outside the context of the larger medical record. In addition, the American Clinical Laboratory Association (ACLA), the association that represents the larger national reference laboratories, has appeared before the MedPAC to raise a number of concerns about the recommendation:

- Considerable cost and effort would be required for laboratories and hospitals to reprogram computer systems to transmit test results to the billing systems to appear on claims. In most institutions, results are reported electronically and test charges are billed electronically, but there is no interface between those systems that would link results to test charges.
The MedPAC suggests that laboratories would standardize nomenclature for tests using LOINC codes, which are more specific than CPT codes. LOINC codes are not commonly used now, and conversion would be a huge effort.

Not all test results are numeric, and not all tests are reported with a reference range. Lengthy narratives accompany many microbiology and flow cytometry results, for example.

Laboratory values should be interpreted in the light of other information about the patient found in the medical record.

Last but not least, the recommendation would need to be examined in the light of HIPAA’s “minimum necessary” privacy standard.

MedPAC discussed whether it might be better to recommend starting the requirement with a subset of specific test results, but in the end stayed with the recommendation that all test results be reported. It did acknowledge that the recommendation represents “…a complex undertaking” and this included a two to three year transition period for implementation.

ACLA is now briefing key congressional staff about their concerns about the MedPAC proposal. The College of American Pathologists (CAP), along with other physician groups, opposes the MedPAC’s proposal to set aside 1% to 2% of physician payments to be redistributed on the basis of performance.

The MedPAC recommendations have gone to CMS, which will have to decide whether to accept them. If CMS decides to move forward, proposed regulations would be published in the Federal Register for comment. ASCLS will monitor this situation closely and register opinions and submit comments whenever appropriate. Regardless of how one views the concept of pay for performance and its potential to improve patient outcomes and patient safety, this particular proposal seems to be unreasonably burdensome for laboratories to implement, and to have limitations in the validity of the conclusions that could be drawn about physician performance from the raw laboratory data.
Evaluation of Malaria Parasite Screening Procedures Among Sudanese Blood Donors

MOHAMED SIDDIG M ALI, ABDUL GADER MOHAMED YOUSIF, MUSTAFA SALIH MUSTAFA, MALIK HASSAN IBRAHIM

OBJECTIVE: To compare the standard microscopic examination, the polymerase chain reaction (PCR), and the immunochromatography test (ICT) to determine the best method for screening blood donors for malaria parasites in Sudan.

METHODS: A total of 100 blood donors were screened for malaria parasites by standard microscopic technique, ICT, and PCR. Blood films were examined microscopically using standard Giemsa staining techniques. Qurum (Canadian Company) malaria kits were used to perform the ICT. For performing PCR, DNA was extracted using Chelex method and amplified by the moderately repetitive DNA sequence pBRK-1.

RESULTS: Using PCR, a total of 21 blood samples were positive; 8 (38%) of them showed negative blood films and 7 (33%) were negative on ICT. Four blood samples that tested positive by ICT despite a negative PCR and microscopic examination were proved to be false positives. The false negativity of both the microscopic examination and ICT was found to be significant. The sensitivity of microscopy was 61.9% and of ICT was 66.7%, while the specificity of microscopy was 100% and of ICT was 94.9%. When direct microscopy was considered as the standard technique the sensitivity of ICT was 100% and the specificity was 94.3%.

CONCLUSION: Although PCR is more sensitive and more specific, it is unaffordable. Microscopy for malaria when compared to ICT showed similar sensitivity at low cost. However, all human plasmodium species can be detected using the microscopy while only two species (P. falciparum and P. vivax) can be detected by ICT. The detected false positivity of ICT is not inconsequential since this implies the rejection of a greater proportion of blood donations. Therefore, microscopy is considered more suitable for screening Sudanese blood donors for malaria parasites prior to donation at the present time.

RECOMMENDATIONS: To establish a reference malaria diagnosis unit in each blood bank in Sudan as well as to train blood bankers to perform microscopic examinations.

ABBREVIATIONS: ICT = immunochromatography test; PCR = polymerase chain reaction.

INDEX TERMS: donor testing; malaria testing.
Prevention of transfusion-induced malaria depends on the screening of potentially infected blood donors, especially those who provide whole blood or fresh concentrates of erythrocytes, leukocytes, or platelets. However, successful screening for malaria parasites requires experience in the differential characteristics of the various species of the parasite and should constitute a part of primary health care. Microscopic examination of a blood film can be used for the detection of malaria infection in a donor in whom malaria is suspected based on circumstantial evidence. Examination of stained thin blood films can identify the species and give an estimate of the percentage of infected red cells. Examination of the buffy coat and red cells below can help in detecting parasites when they are few in number (5 to 10 parasites/µL), particularly in the late stages, e.g., trophozoites and gametocytes. However, in traditional microscopy, failure to adhere to correct techniques seriously compromises the specificity and sensitivity.

Immunochromatography tests (ICT) employ monoclonal antibodies against histidine rich protein (HRP-2), which is produced by the parasite and released into the circulation. Compared to microscopy, it is simple to perform, does not require the use of special equipment, and is faster with low variation between users. Moreover, it can detect asexual parasites and young gametocytes with reasonable sensitivity and specificity (>90%). Serological methods cannot replace the demonstration of parasites in the blood as far as diagnosis of symptomatic patients is concerned. In countries where the disease occurs, antibody tests are unable to distinguish between active and past infection and they have only a limited value in the clinical diagnosis of malaria.

During the past few years, the polymerase chain reaction (PCR) has become a major diagnostic and research technique. It is reliable for the detection of parasites present at low concentration in blood or serum samples. Further, the use of PCR could be 100 times more sensitive than the microscopic examination of thick blood films.

The present study is intended to determine the selection of the best laboratory procedure that can be applied for screening blood donors for malaria parasites in Sudan since such studies have not been conducted previously.

MATERIALS AND METHODS
The design of the study is descriptive, cross-sectional, and facility based. It was conducted in the Ahmed Gasim Hospital (Khartoum North, Sudan) among a total number of 100 blood donors screened for malaria parasites by ICT, PCR, and standard microscopic technique. Blood samples were collected (1 mL from each), processed with EDTA anticoagulant (1.5 mg), and then immediately used to prepare blood films and perform ICT. Three spots of each tested blood sample (50 µL each) to be used for the PCR were stored dry (on #3 filter paper) at -20 °C.

From the blood collected from each donor, duplicate thick and thin blood films were prepared, stained by Giemsa stain, and examined microscopically as the standard methods.

The absolute number of parasites (number/µL), was estimated in positive thick blood films by counting the recognized malaria parasites against 200 white blood cells according to the following formula:

\[
\frac{\text{# of parasites counted} \times \text{total WBC}}{\text{# of leukocytes counted} (200)}
\]

Qurum (Canadian Company) ICT malaria kit was used. This kit has been designed for the detection of P. falciparum in whole blood. It employs monoclonal antibodies against HRP-2 that is secreted by the parasite. Captured monoclonal antibodies are immobilized on a nitrocellulose membrane strip. When P. falciparum antigen is present in lysed whole blood, it binds antibodies as the blood migrates along the test strip. Colloidal gold particles are coated with these antibodies to form a sandwich resulting in visible red line. Performance of the test and interpretation of results were conducted as directed by manufacturer instructions.

For performing PCR, DNA was extracted from the filter paper using Chelex method. One of the three blood spots was cut using a sterile blade and put in a 1.5 mL Eppendorf tube containing 1 mL of 0.5% saponine in freshly prepared 1 x phosphate buffer saline (PBS) and then incubated over-night at 4 °C (hemoglobin is released into the wash leaving the DNA of the parasite on the paper).

The tubes were spun for one minute at 13 x 10^3 rpm and the supernatant fluid was removed. One mL of PBS was added and spun again for one minute; the supernatent was also removed. Fifty µL of PCR quality water and 50 µL of 20% Chelex mixture were added to each tube and then boiled for ten minutes.
The tubes were centri centrifuged for one minute to pellet Chelex and debris. The DNA supernatant was taken off and transferred into a new sterile 0.5 mL Eppendorf tube.

DNA amplification was performed using the moderately repetitive DNA sequence pBRKl-14 forward, 5'-CGC TACATATGCTAG TTGCCA GA C-2' and reverse 5'-' CGTGTA CCATA CATCCTACCAAC-3' that amplifies a 206 base pair sequence. The PCR product was run in a gel electrophoresis tank (1.5% agarose gel) with the addition of ethidium bromide solution (0.5 mg/mL) and DNA molecular weight marker (fraction VI) in a parallel well.

Data were analyzed by the computer using the Statistical Package for Social Sciences (SPSS). Chi-square and Fisher exact tests were used for comparison and correlation between proportions.

The sensitivity of the test is the proportion of positives, corresponding to the positive result obtained by the standard test. However, specificity of the test is the proportion of negatives corresponding to the negative result obtained by the standard technique. Sensitivity, specificity, false negative rate, and false positive rate can be calculated using the following formulas and Table 1:

\begin{align*}
\text{Sensitivity} &= \frac{e}{e + f} \\
\text{Specificity} &= \frac{h}{g + h} \\
\text{False negative rate} &= 1 - \text{Sensitivity} \\
\text{False positive rate} &= 1 - \text{Specificity}
\end{align*}

RESULTS
Out of the 100 randomly selected blood donors, 13 samples (13%) were positive by microscopy as well as by ICT and PCR, with 75 samples (75%) being negative throughout. Twelve blood samples (12%) showed variable results.

Comparison between the results of PCR and ICT of the examined blood samples applying PCR as the standard technique is shown in Table 2. Using PCR, a total of 21 blood samples were positive; of them only 14 blood samples tested positive using ICT. Four blood samples tested positive by ICT despite a negative PCR and microscopic examination; therefore, these proved to be false positives. The false positive of the ICT was found to be statistically insignificant ($p > 0.05$).

Seven (33%) positive PCR blood samples were negative by ICT. These false negative ICT results were found to be highly significant ($p < 0.001$).

Applying PCR as a standard technique, the sensitivity of ICT was 66.7% while the specificity was 94.9%. The false negative rate was 33.3% and the false positive rate was 5.1%. Comparison between the result of PCR and microscopic examination of the donors' blood samples applying PCR as the standard technique is shown in Table 3.

Using PCR as the standard technique, a total of 21 blood samples were positive; 8 (38%) of them showed negative blood films. This false negativity of the microscopic examination was found to be highly significant ($p < 0.001$).

<table>
<thead>
<tr>
<th>Tested technique</th>
<th>Positive (D+)</th>
<th>Negative (D-)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (T+)</td>
<td>(e)</td>
<td>(g)</td>
<td>(e + g)</td>
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<tr>
<td>Negative (T-)</td>
<td>(f)</td>
<td>(h)</td>
<td>(f + h)</td>
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<tr>
<td>Total</td>
<td>(e + f)</td>
<td>(g + h)</td>
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</tbody>
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Table 2. Comparison between the results of PCR and ICT

<table>
<thead>
<tr>
<th></th>
<th>PCR Positive</th>
<th>PCR Negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>ICT positive</td>
<td>14</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>ICT negative</td>
<td>7</td>
<td>75</td>
<td>82</td>
</tr>
<tr>
<td>Total</td>
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<td>79</td>
<td>100</td>
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$p > 0.01$

<table>
<thead>
<tr>
<th>Tested technique</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic examination</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>79</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>79</td>
<td>100</td>
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</table>

$p > 0.01$
Applying PCR as a standard technique, the sensitivity of microscopic examination was 61.9% while the specificity was 100%. The false negative rate was 38.1% and the false positive rate was zero. Comparison between the result of microscopic examination and ICT of the same blood samples applying microscopy as the standard technique is shown in Table 4.

Using microscopic examination, a total of 13 blood samples were positive; 5 (38.5%) of them were ICT negative. Only one donor’s blood sample (7.7%) was found positive by ICT and PCR despite a negative blood film.

The false positive ICT (the other four positives; 22.2%) which were negative by blood films, representing the difference between microscopy and ICT, were found to be insignificant ($p >0.05$).

Applying microscopic examination as standard technique, the sensitivity of ICT was 100% and the specificity was 94.3%. The false positive and false negative rate was 5.7% and zero respectively.

**DISCUSSION**

Transfusion-induced malaria continues its resurgence throughout much of the tropics and subtropics. Successful control or eradication measures must include strategies directed to prevent transmitting malaria parasite infected blood to patients. Therefore, it is mandatory to establish an effective technique for screening blood donors for the malaria parasite. Different techniques were compared to determine the best method of screening donors that can be easily and rapidly applied in Sudan.

Since PCR is highly specific and highly sensitive for positive and negative results respectively (100 times greater than staining technique), it can be used as a standard technique to avoid false negatives and positives. In this study, PCR was adopted as the standard technique. It is worthwhile to mention that during this investigation, the difference between PCR and both ICT and direct microscopy was still significant, although PCR has the drawbacks of being costly and requiring strict laboratory conditions to avoid contamination that can lead to false positive results.

In malaria-endemic areas, the use of ICT is known to be rather deceptive when used for investigating symptomatic patients for the presence of malaria parasite in their blood. False positive results can be obtained even 14 days after clearance of the parasites by treatment or by the immune system.

This study showed that sensitivity of the ICT versus direct microscopy was 100%, which corresponds to the results obtained by Singh in India, Cavallo in France, and Gaye in Senegal. However, all of them confirmed the false positivity of ICT within variable periods after infections. These reports are in close agreement with the findings of this study; four false positive tests were detected. The false positive results are important since this implies that a greater proportion of blood donations will be rejected to ensure the prevention of malaria transmission by blood transfusion. Therefore, ICT seems to be not reliable for screening Sudanese blood donors for malaria parasite since whether the parasite is present or not, persistence of HRP-2 will result in false positivity. Further, only ICT designed for the detection of only two species ($P. falciparum$ and $P. vivax$) is available, whereas transfusion induced malaria due to the other human plasmodium species ($P. malariae$ and $P. ovale$) is not uncommon, particularly $P. malariae$ because of its chronicity. The ICT test will be falsely negative when the latter species are present.

The standard microscopic examination technique for malaria parasites enables the detection of all four human plasmodium species. It also allows distinction between species and stages of infestation and the capability of determining parasitaemia which may produce some new epidemiological and parasitological aspects providing some suggestions for eradication; moreover, it is cheap, relatively rapid, readily available, and easy to perform.

When compared with ICT, microscopic examination shows similar sensitivity (no statistical difference; $p >0.05$). Hence, microscopy of malaria is more suitable for screening blood donors in Sudan. This is in agreement with Wilairat’s findings that microscopic diagnosis of malaria is still more than adequate.

<table>
<thead>
<tr>
<th>Table 4. Comparison between the result of microscopic examination and ICT of the same blood samples applying microscopy as standard technique</th>
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<tbody>
<tr>
<td><strong>Microscopic examination</strong></td>
</tr>
<tr>
<td>ICT positive</td>
</tr>
<tr>
<td>ICT negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>$p =1.20$</td>
</tr>
</tbody>
</table>
in the routine investigation of individual cases, in spite of the fact that many authors have not favored the use of this method because of the amount of time and expertise required.\textsuperscript{14}

There is still no international consensus for the exact definitions of what constitutes high, intermediate, or low parasitaemia; undoubtedly, most of the parasite densities that were encountered in our study were relatively low. However, the minimum number of parasites transmitting malaria via blood transfusion may vary among individual recipients; a single parasite can transmit the disease in mice.\textsuperscript{13} Even if a single parasite can be detected in a thick blood film, which is equivalent to 4 $\mu$L of blood, more than hundreds of thousands of parasites might escape in a full unit of blood (450 mL).

This signifies the importance of the reliable testing of blood donors for malaria parasite to minimize, though never completely eliminate, the risk of malaria transmission by blood transfusion. Moreover, the hazards of transfusion malaria are serious, and justify prior testing of blood donors for malaria even by advanced expensive techniques. These techniques will surely reduce the false negative results and hence minimize the risk of transfusing infected blood.

The standard microscopic identification technique of malaria parasites is ideal to be applied at the present time until the development of more feasible application of PCR. The establishment of a reference malaria diagnosis unit in each blood bank as well as trained blood bankers is necessary.

CONCLUSION

The hazards of transmitting malaria through blood transfusion are serious thus justifying the use of expensive techniques for testing blood donors. Transfusion of blood from malaria parasite infected donors to patients will result in transfusion-malaria; screening donors for malaria prior to donation will undoubtedly reduce this risk. Microscopy is much cheaper than both ICT and PCR; in addition, it enables the detection of all human plasmodium species. Therefore, it is the best technique to be adopted for the control of transfusion-induced malaria in the different regions of Sudan until the feasibility of using PCR technology is improved.

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REFERENCES

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

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The CLS Program at the University of Cincinnati introduced an AS to BS degree completion distance learning track (CLS DL) in June 2004. The track was designed to provide working laboratory professionals with an associate degree the opportunity to earn a bachelor’s degree while continuing full- or part-time employment, and with minimal disruption in their lives. Previous laboratory and life experiences were considered in curriculum development, so students enter the program with advanced standing that includes complete fulfillment of the university’s general education requirements. The curriculum includes upper division science content, didactic courses, and advanced clinical experiences that are completed in each student’s community. The courses are taught using an interactive distance learning course model that includes audio and video PowerPoint presentations, regular discussions between the instructors and the students, and frequent learning assessments. The students matriculate through the curriculum in a learning community of 20 students that provides a natural support system and study group environment. Fifty-four students were admitted into the first quarter; 94% were retained. One hundred three new students were admitted into the second quarter. Funding, development, resources, the course model, and the curriculum will be discussed and sample course materials will be presented. Student demographic, assessment, and retention data will be shown. Preliminary data suggest that the distance learning course model and curricular structure utilized in the CLS DL track will successfully provide laboratory professionals who are not able to attend a traditional program with the means to continue their education and advance their careers.

Evaluating Medical Students: It’s Not Always “THE LAB’S FAULT!”

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Clinical laboratory scientists (CLSs) often complain that other healthcare providers, in particular physicians, do not recognize our educational level and underutilize our expertise. In addition, clinicians have little or no knowledge of the effects of pre-analytical errors, especially those resulting from improper specimen collection. As a result, when laboratory values do not match clinical diagnosis – it’s the lab’s fault! This project, directed by the Clinical Laboratory Science Program faculty, was part of a medical school course for students entering their junior year. We integrated phlebotomy, clinical data, and laboratory data to expose medical students to the value of laboratory data and the expertise of CLS. The class was divided into five groups, each of which met for a half day – one-half of the time was spent in small work groups, the remainder in phlebotomy. Twelve scenarios were developed covering reflex testing and sources of pre-analytical error in

Development and Implementation of an Innovative MLT/CLT to MT/CLS Articulation Program Using Synchronous and Asynchronous Delivery Formats

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It is well established that there is a shortage of certified medical technologists/clinical laboratory scientists (MTs/CLSs). The problem identified in this study is that there are limited programs that address the development of a curriculum format that is accessible by working medical laboratory technicians/clinical laboratory technicians (MLTs/CLTs) to earn a bachelor’s degree. In 1996, a new model of curriculum delivery using synchronous and asynchronous formats to deliver the didactic components was developed and implemented by Old Dominion University. The program utilizes an interactive, televised, asynchronous weekend format to deliver courses to distant sites. The work sites of MLTs/CLTs provide the clinical component of the program. This study was a retrospective cross-sectional comparison of the scores of traditional and weekend students (n = 97) on the American Society of Clinical Pathology (ASCP) national certification examination over a 4½ year period beginning in 1998. In every comparison there was no difference, i.e., p<0.001, between the scores of traditional and weekend students on the ASCP examination. Furthermore, the scores of traditional and weekend students in four of the six subject areas (hematology, blood bank, microbiology, and chemistry) exceeded not only the national average in these subject areas, but also the cumulative overall score. This suggests that the Old Dominion University MLT/CLT to MT/CLS Program, delivered in this innovative format, is effective. Further research needs to be conducted in order to examine the cost/benefit of this type of program as well as other delivery formats.
all laboratory disciplines. Each student group identified the problem in the scenario and determined which laboratory professional could be consulted regarding questions about test ordering or interpretation. Students then presented the results to other members of the class. CLS faculty moderated the sessions. CLS students assisted with venipuncture. Medical students took a pre- and post-test that measured knowledge of laboratory testing. The difference between means of the pre- and post-test was significant. Comparison of the means of the pre- and post-test showed a 30% improvement in scores. Session evaluations were favorable with many students suggesting additional time for the activities.

**Fostering the Development of Expertise in Clinical Laboratory Scientists (CLSs)**

*Janet Hudzicki PhD MT(ASCP)SM, Kansas State University, Manhattan KS.*

The development of expertise is a phenomenon that is little understood. Although there is a body of research that examines the characteristics of experts, and compares experts to novices, the literature on the actual transition process lacks depth. This research describes an investigation of the transition from novice to expert in the clinical laboratory science community of practice using a phenomenological approach. The sample selection process consisted of soliciting names of expert CLSs from the members of the Clinical Laboratory Managers Association. The potential participants were randomly selected from the submitted names and asked to participate in the study. Data were collected from 11 participants by semi-structured interviews. The constant comparative method was used to analyze the interview transcripts. Four factors were determined to be essential to the transition process: Self-directedness in learning, storytelling, mentors and mentoring, and reflection. In addition, the transition from novice to expert requires being part of a vital, robust community of practice. Recommendations for helping novices with this transition include establishing a mentoring program for students and new employees, encouraging storytelling among laboratory personnel, and providing tools that will encourage reflection. This research has the potential to impact the education and training of CLSs, the enculturation of novice CLSs into the profession's community of practice, and the development of expertise in CLSs.

**Integrating Education of MT/CLS Students and CP Residents in a Single Course**

*Nancy Goodyear PhD CLS(NCA), University of Washington, Seattle WA.*

It is unusual for undergraduate MT/CLS students to have the opportunity to interact on an equal basis with clinical pathology (CP) residents. Pathology residents in the University of Washington Department of Laboratory Medicine begin their CP training with a three-month structured core course covering all areas of the clinical laboratory. The microbiology portion begins with eight laboratory sessions taught by an experienced clinical technologist or a microbiology post-doctoral fellow. Following this introduction, the residents join the MT Program clinical microbiology laboratory class for three to four weeks. Following the core, they rotate through the clinical labs, including at least six weeks in microbiology. Many residents have no clinical laboratory or microbiology background; although they don’t need to develop technical expertise, working up the same specimens as the MT/CLS students, from plating to final report, helps them to understand the testing performed in microbiology. Interactions between MT/CLS students and residents help both groups recognize the critical role that each plays in healthcare, and appreciate the expertise and limitations of each group’s training. MT/CLS students assist residents with laboratory procedures and colony morphology. Residents bring human organs from the Department of Pathology teaching organ collection and give demonstrations reviewing normal and abnormal anatomy, especially as it applies to infection. In addition to providing an opportunity for interprofessional interactions, combining MT/CLS students and CP residents in the same microbiology course consolidates teaching workload, improves resource utilization, and provides an opportunity for MT program faculty to contribute to a larger educational mission.

**Moving from Traditional to Online Delivery: Creating a Hemostasis Course That Promotes Student Participation**

*Margaret Fritsma MA MT(ASCP)SBB, University of Alabama at Birmingham, Birmingham AL.*

University clinical laboratory science programs are moving in the direction of distance learning to make courses more accessible to students. While there are advantages and disadvantages to both classroom-based courses and online courses, most undergraduate students prefer classroom-based learning. However, online delivery can be a more convenient and cost-effective option. This course on hemostasis provides an example of how online delivery can be effective in promoting student participation and engagement. The course includes interactive modules, quizzes, and case studies that help students apply their knowledge to real-world scenarios. Additionally, the use of multimedia resources, such as videos and animations, can make the learning experience more engaging and memorable. Overall, the course has been well-received by students, with many reporting increased understanding and retention of the material.
based lectures to online delivery. The challenge to educators is to design online courses that engage students, accommodate various learning styles, incorporate a variety of learning activities, and build in accountability and student participation. A hemostasis course is described in which course content is placed online, with classroom follow-up. Each week's lesson material is placed online in the form of lesson objectives, PowerPoint slides with lecture audio, a Microsoft Word handout with slides accompanied by written text of the lecture, and assignments and supplemental material. There is one classroom meeting each week, which consists of a short quiz on the online material, an interactive discussion (no lecture) of the more difficult concepts and implications from the lesson material, a question and answer (Q&A) session, and various group activities. Examples of group activities include impromptu group presentations of short topics, games, student bowl competition, a Protein C Pathway skit, and case discussions. Each student is assigned one module to narrate the audio portion of the online lesson from the instructor's scripted text, and to lead the classroom Q&A session on his/her topic. Student interest and feedback is positive, and will guide future development of the course. Exam scores have shown improvement in grades over the previous course offered in a more traditional format.

A Novel Consortium Model for Delivering Clinical Laboratory Programs to Rural Regions
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Workforce problems including severe shortages of clinical laboratory technicians (CLTs) in surrounding rural regions and shortages of histology technicians (HTs) in both rural and urban regions, coupled with a state mandated initiative (“Closing the Gaps”) to increase student participation and success within higher education institutions in the State of Texas, prompted the Clinical Laboratory Science (CLS) Department at this institution to generate and investigate alternative program and curriculum models in order to address these problems. The chosen solution was to design a unique consortium model that represents a novel cooperation between the two-year and four-year higher education sectors, and is supported by a unique curriculum design that facilitates access and participation. Four key outcomes of the resulting consortium model are that it 1) delivers two high need laboratory science programs (CLT and HT) and thus contributes to the “Closing the Gaps” initiative in three of four key objective areas; 2) utilizes a large community college partnership base with established clinical affiliations to serve a large, predominantly rural geographic region and to maintain sufficient student numbers for program viability; 3) realizes cost efficiencies by capitalizing on an existing infrastructure of resources and expertise already in place to support CLS programs; and 4) facilitates laboratory science student articulation between professional levels. This novel consortium model may be applied to institutions nationwide that are facing similar problems with diminishing state funding, program viability concerns due to low student numbers, workforce shortages, and increasing demands for student access, participation, and success.

The Relationship of Proficiency Test Performance to Personnel Credentials of Laboratory Testing Personnel
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Performance on proficiency test (PT) surveys provides an objective and consistent evaluation of laboratory quality. The goal of the study, a retrospective review of existing PT results performed at Virginia Commonwealth University Health System laboratories, was to determine the relationship of PT performance to the personnel credentials of the laboratory testing personnel. Predictor variables included the practitioner’s major area of study, degree, certification, and years of laboratory experience. The study group consisted of 185 testing personnel. There were 3389 proficiency-testing results of which 3306 were graded acceptable (97.6%), 36 were unacceptable (1.1%), and 47 were not graded (1.4%). For those results performed by a single practitioner (n = 3266), the core laboratory performed 3161 (96.8%) of the PT results with 30 unacceptable (0.95%) results. The satellite laboratories staffed by non-laboratorians performed 105 (3.2%) of the results and 6 (5.7%) were unacceptable. Logistic regression analysis of the full model, with all predictors included, showed statistical significance ($\chi^2 = 18.581, p = 0.010, df = 7$) for years of experience and level of educational degree. Individuals with less than two years experience were over five times more likely to produce an erroneous result when compared to those with 20 years of clinical experience. Study limitations included the use of a single institution and incomplete demographics for six testing personnel who were responsible for two (5.5%) of the unacceptable PT results. As the laboratory workforce shortage intensifies, the performance of laboratory personnel with limited years of clinical experience or those lacking an educational degree may be important.
The Use of Games to Review in a Clinical Microbiology Class
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A method of reviewing material prior to tests in clinical microbiology was desired that did not involve reteaching. To meet this objective, games were developed such as “You Make Me Sick”, “You Grow On Me”, “Jeopardy”, “Who Wants to be a Microbiologist?”, “Go Streaking”, “Microbiology Team Competition”, “Enterobactriaceae Squares”, and “BACT” (bingo). The games provided opportunities for students to review material in a non-threatening, interactive, sometimes competitive, and fun way. The use of games was an effective means of reviewing/reinforcing material as indicated by students’ mean scores on the four exams (82.6, 84.7, 87, and 86.1). On an evaluation, 100% of students responded that they enjoyed playing games, the games were a beneficial way to review for tests, the games helped them learn important facts/concepts, and the games were relevant to exam questions. All the students liked team games better than individual games. The games that students felt were most helpful in preparing for exams and learning facts and concepts were “You Grow on Me”, “You Make Me Sick”, “Jeopardy”, and “Who Wants to be a Microbiologist?”. Students’ written comments about using the games were very positive and included such statements as “The games helped me a lot,” “I really liked the games. They were very helpful in preparing for exams,” and “The games were very good review tools.” Based on these results I will continue using these games and others in the microbiology class as well as in the immunology class that I teach.

TECHNOLOGY DEMONSTRATIONS

Demonstration of Microsoft Producer for the Development of High Quality Recorded Lectures Based on PowerPoint Presentations
Scott Wright MS, Weber State University, Ogden UT.

For the past three years, the Clinical Laboratory Sciences Department at Weber State University has offered both CLT and CLS degrees online. Presently, one third of the courses offered through the Department deliver course lectures to the online student on a CD. The CD is mailed directly to the student prior to the beginning of the semester, eliminating problems associated with streaming video delivered over the Internet.

The lectures are created by first writing a script which is then recorded and synchronized to PowerPoint slides using a program called Microsoft Producer for PowerPoint 2003. This technology demonstration will involve two computers; the first to play various examples of recorded lectures, and the second to demonstrate the relatively simple steps involved in creating high quality recorded lectures using the Microsoft Producer software (available for free at www.microsoft.com). The results of a student survey will be available describing the popularity of the recorded lectures, the ease of use, and convenience for the online student.

Integrating Learning Objects into Clinical Microbiology Teaching Materials
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Time to produce educational content is a major concern when planning lessons for CLS/CLT students. Learning objects (LOs) are an approach to producing content in which the instructional material is broken down into ‘bite size’ chunks. These chunks can be independently created, maintained, reused, and pulled apart and then stuck back together into many different forms like Lego toys. LOs are a high quality technical resource for lectures, reviews, or tests that can be used to structure a lesson individually or strung together to create interactive content. LOs may include a combination of pictures, graphics, animation, video, audio, and text components. Because they are visual in nature, they are an asset for the development of lesson structure in both distance learning and computer-assisted learning environments. The use of LOs can reduce the preparation time for lectures, examinations, and remediation materials, freeing instructors to focus on other tasks. The University of Texas Medical Branch Clinical Laboratory Science Program partnering with the University of Nebraska Medical Center Division of Medical Technology received a Fund for Improvement for Postsecondary Education Grant to create LOs and disseminate them via an online repository. The current focus of this repository is microbiology and provides instructional content on biochemical reactions, organism identification, panel selection, and gram stain quality control. The accompanying technology demonstration will provide the actual LOs and demonstrate the sequencing of LOs to form a cohesive lesson.
Membranoproliferative Glomerulonephritis
Type II in a 10-year-old Girl

MARThA E TIBBS, SHARON P ANDREOLI, CARRIE L PHILLIPS

The clinical course of a 10-year-old female patient who presented with hematuria, proteinuria, and hypertension is described. Four months after being diagnosed with acute glomerulonephritis, the child was referred to a pediatric nephrologist due to persistent hematuria and unresolved proteinuria. A renal biopsy was performed due to the persistent urinary abnormalities and a family history of renal failure. The renal biopsy demonstrated pathological findings characteristic of membranoproliferative glomerulonephritis type II. The child was treated with an antihypertensive agent and steroids. Despite poor prognostic clinical and pathological features, she has minimal urinary abnormalities, normal renal function, and normal blood pressure on antihypertensive medication six years after the diagnosis of membranoproliferative glomerulonephritis type II.

ABBREVIATIONS: C3 = complement component 3; GBM = glomerular basement membranes; Ig = immunoglobulin; MPGN = membranoproliferative glomerulonephritis.

INDEX TERMS: C3 nephritic factor; complement; dense deposit disease; membranoproliferative glomerulonephritis; prednisone.


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The nephrotic and nephritic syndromes are clinical consequences of structural injury to the renal glomerulus, a vascular filter that clears the blood of waste products. Laboratory testing is required to distinguish the two syndromes. Proteinuria, the major clinical sign of nephrotic syndrome, results from altered permeability of the glomerular filtration barrier due to perturbations in visceral epithelial cells (podocytes). Nephrotic syndrome in children is the clinical manifestation of podocyte injury most commonly due to minimal change disease. Other causes include focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, or membranous glomerulopathy. Children with nephritic syndrome usually present with gross hematuria that may be accompanied by hypertension and variable degrees of proteinuria. Inflammatory processes that target glomeruli, termed acute glomerulonephritis, alter capillary wall integrity, and allow red blood cells to leak into urine. For a more comprehensive analysis of acute glomerulonephritis, the reader is directed to the review by Vinen and Oliveira. 1 Hematuria occurs in IgA nephropathy, hypocomplementemic glomerulonephritis, and hereditary renal disease (Table 1). Children who present with overlapping features of both nephrotic and nephritic syndromes should undergo thorough serological testing to narrow down the differential diagnosis. When hematuria and proteinuria are accompanied by hypocomplementemia (Table 1), a renal biopsy may be required to determine the underlying etiology and to guide therapeutic management. If the acute process is untreated, these patients risk progression to chronic glomerulonephritis and may require renal replacement therapy such as dialysis or transplantation (for an excellent review, see Coppo and Amora 2004). 2

Membranoproliferative glomerulonephritis (MPGN), or mesangiocapillary glomerulonephritis, is caused by an abnormal immune response leading to antibody deposition in...
Table 1. Differential diagnoses of pediatric patients with hematuria and proteinuria

Glomerulonephritis
- Hypocomplementemic glomerulonephritis
- Postinfectious glomerulonephritis
- Membranoproliferative glomerulonephritis, types I, II, and III
- Systemic lupus erythematosus
- Nephritis of chronic infection
- IgA related glomerulonephritis
- Henoch-Schönlein purpura
- Systemic lupus erythematosus
- IgA nephropathy
- Membranous glomerulonephritis

Hereditary renal diseases
- Alport syndrome (hereditary nephritis)
- Sickle cell nephropathy
- Autosomal dominant polycystic kidney disease
- Autosomal recessive kidney disease

The membrano- portion refers to the histological observation of glomerular capillary wall thickening due to glomerular basement membrane (GBM) alterations. Increased glomerular cellularity suggests there is a proliferative basis to the glomerulonephritis. Patients with MPGN typically experience a decline in renal function as a consequence of inflammation and structural alterations of the kidney. MPGN is subtyped into three categories based on the pathway of serum complement activation and altered renal morphology resulting from deposition of immunoglobulin and complement in glomeruli. All three subtypes of MPGN are characterized by a decreased serum C3 level in 80% to 90% of patients and the low serum complement level is an important clinical tool in the preliminary diagnosis of glomerulonephritis (Table 1). MPGN type I is characterized by the classical pathway of complement activation and immune deposits along the subendothelial aspect of the GBM. MPGN type II, or dense deposit disease, is characterized by ‘dense’ ribbon-like immune deposits within the GBM, alternative complement pathway activation, and circulating C3 nephritic factor. MPGN type III is characterized by subendothelial, subepithelial, and mesangial immune deposits with activation of the alternative complement pathway.

CASE STUDY
In September 1997, a 10-year-old girl was seen by her family physician for a one week history of dysuria and brownish-colored urine. Upon physical examination, the patient was afebrile with a blood pressure of 124/92 mmHg. The urine dipstick was positive for protein. Urine microscopy showed numerous white and red blood cells. The child was diagnosed with acute glomerulonephritis and was prescribed a
ten-day course of amoxicillin. In January 1998, the child returned to her physician’s office with bronchitis. At that time she had a history of gross hematuria, her urine was brown in color, and the urine dipstick was positive for protein and blood. A ten-day treatment of clarithromycin was prescribed, blood tests were drawn, and the child was referred to a pediatric nephrologist.

At the pediatric nephrology clinic, the patient’s only symptom was intermittent abdominal pain. Blood pressure was 130/90 mmHg and the physical exam was otherwise unremarkable. Urinalysis demonstrated persistent hematuria and proteinuria, the serum complement levels were normal, and the 24-hour total urine protein excretion was substantially elevated at 2,420 mg (normal is less than 150 to 250 mg/day). Additional laboratory results conducted on serum, summarized in Table 2, included decreased albumin, borderline low total protein, and elevated cholesterol and alkaline phosphatase. A renal ultrasound showed bilateral enlargement of the kidneys. Serology typical of postinfectious glomerulonephritis (Streptozyme®) or nephritis associated with systemic lupus erythematosus (anti-nuclear antibody) was negative. Her family history was remarkable for two maternal uncles with kidney disease each requiring a renal transplant while her mother’s urinalysis was negative for blood. Her nephrologist suspected the child had a form of glomerulonephritis, likely IgA nephropathy, but was concerned about the possibility of hereditary nephritis. A kidney biopsy was performed to establish a definitive diagnosis.

**The renal biopsy**

The renal biopsy specimen contained over 60 glomeruli by light microscopy. Ten percent of the glomeruli were obsolescent or totally scarred. The remaining glomeruli showed lobular accentuation of the glomerular tufts secondary to diffusely increased cellularity, thickened capil-
lary loops, and excess accumulation of mesangial matrix (Figure 1). The glomerular hypercellularity was due to increased mononuclear cells in the mesangium. Jones’ silver stain showed thickened and focally duplicated GBM (‘tram-tracks’) with increased mesangial matrix (Figure 1, inset). Cellular crescents, or extracapillary proliferation in Bowman’s space, were present in 10% of the glomeruli. Interstitial edema was apparent. The tubules and vessels were histologically unremarkable.

Direct immunofluorescence examination of the renal biopsy specimen revealed strong immunofluorescence staining for complement C3 along the glomerular capillary loops (3+ to 4+ intensity on a scale of 0 to 4+) accompanied by weaker staining in the mesangium (1+ to 2+) (Figure 2). Staining of capillary loops and mesangium with immunoglobulins IgG, IgA, and IgM was negative to weak (0 to 1+). Staining with complement C1q and fibrinogen was negative.

One glomerulus was examined by electron microscopy. Ultrastructural examination revealed numerous dense, elongated ribbon-like deposits along the lamina densa of GBM (Figure 3). Podocyte foot processes were effaced and the mesangial matrix was increased.

Clinical course
The renal biopsy results were consistent with MPGN type II or dense deposit disease. Based on the results of the renal biopsy, the child received six pulses of intravenous methylprednisolone on an every other day schedule. This was followed by oral prednisone, 60 mg every other day. For hypertension, the patient was prescribed an angiotensin-converting enzyme inhibitor, enalapril, 2.5 mg/day. Six months after the kidney biopsy and initiation of therapy, her serum albumin had increased to 3.8 mg/dL, her 24-hour total urine protein excretion had decreased to 760 mg and she had no further episodes of gross hematuria. She was treated with reducing doses of alternate day prednisone for the next three years. During this interval, her serum albumin and creatinine remained normal, her microscopic hematuria resolved and her 24-hour total urine protein excretion decreased to less than 500 mg per day. Six years after the diagnosis of MPGN type II and nearly three years after discontinuation of prednisone therapy, she continues to have normal renal function and normal blood pressure, no microscopic hematuria, and minimal proteinuria as demonstrated by a urine protein excretion of less than 500 mg per day.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS TYPE II
Clinical findings
MPGN is a common childhood glomerulonephritis that usually progresses to chronic renal failure. Of the three subtypes of MPGN, type II is observed less frequently, accounting for about 20% to 30% of the cases of all MPGNs. Adults and children affected by MPGN type II are typically less than 20 years old with a median age of 11.5 years. Many children ultimately found to have MPGN originally receive medical attention due to asymptomatic hematuria and proteinuria. MPGN may present as either acute nephritic syndrome or nephrotic syndrome, with or without gross hematuria. Hypertension occurs in some patients due to water and sodium retention, increased production of renin by the kidney, and other complex mechanisms that regulate blood pressure. In some cases, a patient may present with findings typical of poststreptococcal acute glomerulonephritis; however, if resolution of the symptoms of postinfectious glomerulonephritis does not occur within six to eight weeks then other glomerulonephritides need to be considered (Table 1). Since the patient described in this report had persistent urinary abnormalities for a period of several months, it became likely that she did not have postinfectious glomerulonephritis and she underwent a kidney biopsy for the determination of a definitive diagnosis.

Laboratory findings
In a patient with MPGN, the major findings on urinalysis are hematuria and proteinuria. Proteinuria may be pronounced and therefore lead to hypoalbuminemia, hyperlipidemia, and edema: all features characteristic of nephrotic syndrome. Edema may arise as a result of decreased oncotic pressure resulting in fluid leaking from the intravascular space (within the blood vessel) into the tissues when serum protein levels are decreased due to urinary losses. At presentation, the patient’s blood urea nitrogen and creatinine levels are usually normal to slightly elevated, unless a rapidly progressive glomerulonephritis is evident. A normocytic, normochromic anemia may also be present.

In all three subtypes of MPGN, a major laboratory finding is hypocomplementemia characterized by low levels of C3, which is important to help characterize and formulate a provisional diagnosis in a child with hematuria and proteinuria. Depressed serum C3 is related to increased catabolism and decreased synthesis of complement. The C3 nephritic factor, NF, is present in 30% to 75% of cases. C3 nephritic factor is an IgG autoantibody that stabilizes
Pathological findings
The kidneys show no gross abnormalities that reflect the pathologic process in MPGN type II, although some patients may have bilateral renal enlargement as is typical in most patients with any form of glomerulonephritis. To separate the types of MPGN, ultrastructural examination is required to demonstrate the glomerular alterations. In MPGN type II, transmission electron microscopy shows sausage-shaped or ribbon-like osmiophilic (electron dense) deposits within the lamina densa of the GBM. Tubular basement membranes may be widened by similar deposits. The deposits occupy short segments in the glomeruli that are distributed irregularly in less severe cases. The dense deposits may also occur in the mesangium.

When examined by brightfield microscopy, the hematoxylin-eosin stain imparts a lobular accentuation to the glomerular architecture due to capillary wall thickening and variable mesangial hypercellularity. Occasionally crescents may involve a portion of the glomeruli. Mesangial sclerosis, or scarring, is more obvious with disease progression. The source of glomerular capillary wall thickening is revealed by periodic acid-Schiff (PAS) or Jones’ silver stains that densely label duplicated basement membranes or 'tram-tracks' and impart weaker staining of the dense deposits. Thioflavin T stain gives a green coloration to the affected basement membranes when using fluorescence microscopy. Similar staining can be observed along Bowman’s capsule and in the mesangia. PAS and Jones’ stains highlight the mesangial sclerosis resulting from expansion of extracellular matrix. Tubular basement membranes may be thickened.

Direct immunofluorescence microscopy shows extensive deposition of C3 within glomerular capillary loops and mesangia. Immunoglobulins are typically scarce; however, if they are present, the immunoglobulins are usually limited to a few glomerular segments.

Pathogenesis
The pathogenesis of MPGN type II is unclear but the primary morphology is dense deposits in the GBM. By unknown mechanisms, glomerular inflammation occurs subsequently. The origin and nature of the dense deposits are unknown.

C3 nephritic factor has been implicated in the pathogenesis of MPGN type II due to the hypocomplementemia. However, the disease progression does not appear to be affected by hypocomplementemia nor C3 nephritic factor.

Prognosis
The indicators of poor prognosis in MPGN type II are hypertension, impaired renal function at time of diagnosis, nephrotic syndrome, and the presence of crescents. The higher the percentage of glomeruli with crescents, the more rapidly renal function deteriorates. Although most patients diagnosed with MPGN type II have no visual complaints, MPGN type II is associated with abnormal retinal function. Dense deposits are observed in the Bruch membrane and the basement membrane of the choriocapillaris. Drusen deposits and retinal pigment epithelial disturbances are characteristic of dense deposit disease retinopathy. These findings are more commonly seen in patients with longstanding MPGN type II. MPGN type II usually progresses slowly to chronic renal failure. Fifty percent of the children will develop chronic renal failure within ten years following diagnosis. Within 20 years of diagnosis, 80% to 90% have chronic renal failure.

Management
Several different therapies have been used, including antiplatelet therapy and immunosuppression. However, to date, there is no universally accepted form of therapy. Most pediatric nephrologists use alternate-day prednisone therapy. Prednisone appears to stabilize renal function and improves disease characteristics; however, it can produce side effects due to drug toxicity. These side effects include stunted growth in children, hypertension, weight gain, Cushinoid features, and mood swings/personality changes. Therapy with pulse intravenous methylprednisolone followed by alternate-day oral prednisone has an improved outcome. The alternate-day regimen either suppresses the immune process underlying glomerular inflammation or decreases inflammation itself to inactivate the disease.

Treatment may vary according to clinical symptoms and laboratory evaluation. The goals of the treatment are to reduce symptoms, prevent complications, and slow progression of the disease. Some children may require dietary restrictions on sodium, fluids, and protein to control high blood pressure, swelling, and accumulation of waste products in the bloodstream. Antihypertensive and diuretic therapy may be needed for treatment of edema and hypertension. Therapy with an angiotensin-converting enzyme inhibitor may be used to decrease urinary protein excretion and slow the
progression of chronic renal failure. To manage chronic renal failure, dialysis or kidney transplantation may eventually be necessary. However, in 50% to 100% of kidney transplant recipients, recurrence of dense deposits in the transplanted kidney may develop. When MPGN type II reoccurs, it leads to graft loss in 10% to 20% of the patients.

SUMMARY
The patient described in this case study presented with hematuria, proteinuria, and elevated blood pressure. Contrary to most patients with MPGN type II, this 10-year-old female had a normal C3 complement level. Without the renal biopsy, this patient would not have been diagnosed with MPGN type II. Although the current therapy has done well in most cases to slow the progression of the disease, there is still a need for the development of a universally effective treatment that does not have significant adverse side effects.

ACKNOWLEDGEMENTS
This report is in compliance with Indiana University School of Medicine’s institutional review board and the Health Insurance Portability and Accountability Act of 1996.

REFERENCES
“Children on the Frontline Against E.coli”:
Typical Hemolytic-Uremic Syndrome

HEIDI ANDERSEN

A thirteen-month old infant presented with classical hemolytic-uremic syndrome (HUS), but with negative cultures for Escherichia coli (E. coli) 0157:H7. HUS is commonly linked to infection with E. coli 0157:H7; however, traditional culture has demonstrated poor sensitivity. Pathogenesis of the organism in HUS involves the production of a Shiga-like toxin (STX), resulting in a triad of symptoms. An early and accurate differential diagnosis, based on patient presentation with acute renal failure, hemolytic anemia, and thrombocytopenia, is critical for supportive treatment and improved prognosis. Patient prognosis is related to the duration of renal failure and dialysis treatment. Research is aimed at improved detection of E. coli 0157:H7 or the STX produced, and future vaccination to eliminate typical HUS.

ABBREVIATIONS: CDC = Centers for Disease Control and Prevention; HUS = hemolytic-uremic syndrome; STX = shiga-like toxin; TTP = thrombotic thrombocytopenic purpura.

INDEX TERMS: hemolytic-uremic syndrome.


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CASE STUDY

In January, a thirteen-month-old Caucasian male presented with progressive diarrhea over a period of two weeks. During this time, medical attention was sought, and the infant was diagnosed with a common childhood diarrhea, suspected to be due to a rotavirus. However, the infant continued with progressive diarrhea and began showing signs of pallor, dehydration, petechiae on his thighs, and decreased appetite. The infant began experiencing episodes of acute abdominal pain with intermittent periods of lethargy. As the diarrhea worsened, one sixteenth of a tablet of Imodium® was given to the infant and he was brought to the local emergency department (ED).

The infant presented in the ED with signs of edema in the extremities from oliguria and acute renal failure. He was catheterized, treated with Lasix® to stimulate kidney function, and given nutritional IV support. Vitals revealed hypertension with a blood pressure of 132/55 (normal blood pressure of a 1-year-old Caucasian male is 102/57) and a temperature of 103.5 °F. As a result, the infant was given Hydralazine® and Tylenol® to reduce his elevated blood pressure and temperature respectively.

The initial laboratory results indicated a diagnostic triad of thrombocytopenia, hemolytic anemia, and acute renal failure (Table 1). Subsequently, the infant was diagnosed with hemolytic-uremic syndrome (HUS). Also, it is important to note that the infant’s increased white cell count and fever supported the diagnosis of the onset of the inflammatory reaction that occurs in typical HUS, with E. coli 0157:H7 suspected as the cause.

QUESTIONS TO BE CONSIDERED

• What is the typical presentation of HUS and the patient population at risk?
• What is the pathogenesis and pathophysiology of typical HUS?
• What is the differential diagnosis of typical HUS?
INTRODUCTION TO HUS

The syndrome now known as HUS, was described in 1955 by Gasser. In 1982, Riley isolated pathogenic Shiga-like toxin producing E. coli (STEC) serotype 0157:H7 from contaminated hamburger. Then, Karmali linked HUS to E. coli 0157:H7 in 1985. Connection of E. coli 0157:H7 to HUS was a major breakthrough in differentiating the mechanisms of the often-confused conditions of thrombotic thrombocytopenic purpura (TTP) and HUS. HUS is currently accepted as the most common cause of acute renal failure in children in the U.S. and is primarily caused by E. coli 0157:H7. The Centers for Disease Control and Prevention (CDC) estimate that with 73,000 E. coli 0157:H7 infections per year in the U.S., 2% to 7% of E. coli 0157:H7 infections result in HUS with the majority of cases occurring in Caucasians less than five years of age. 1 The elderly, adults, and older children have higher mortality rates from HUS than occur in younger children. 2,3 Interestingly, HUS caused by infection with E. coli 0157:H7 is less common in African-Americans. 4 Overall, E. coli 0157:H7 infections costs about 60 lives and $660 million dollars each year, and is now an endemic cause of HUS in the U.S. 1,5,6

Classical HUS is defined as a thrombotic microangiopathy with a diagnostic triad of acute hemolytic anemia with schistocytes, thrombocytopenia, and acute renal failure. However, classical HUS does not always present with a complete diagnostic triad. A major indicator of typical HUS from E. coli 0157:H7 is presentation with a one to eight day acute gastroenteritis prodrome and bloody diarrhea, unless the infection is acquired by a urinary tract infection or a respiratory infection. 7,8 The kidney is the major organ target in classical HUS, but other organs such as pancreas, heart, lungs, and brain may also be involved. 9,12 Other factors supportive of a diagnosis of HUS due to E. coli 0157:H7 include its predominance in the summer months and its potential to occur in outbreaks.

Table 1. Initial laboratory testing of patient on day 1 of hospitalization

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>33</td>
<td>30.8 - 46.6 g/L</td>
</tr>
<tr>
<td>BUN</td>
<td>32</td>
<td>1.8 - 7.1 mmol/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>102</td>
<td>95 - 105 mmol/L</td>
</tr>
<tr>
<td>CO2</td>
<td>17</td>
<td>22 - 27 mmol/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>336</td>
<td>17.7 - 61.9 mol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.7</td>
<td>2.2 - 11.1 mmol/L</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.258</td>
<td>0.340 - 0.480</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>85</td>
<td>96 - 156 g/L</td>
</tr>
<tr>
<td>Platelet #</td>
<td>64</td>
<td>150 - 450 x 10^9/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.3</td>
<td>3.5 - 5.5 mmol/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>131</td>
<td>135 - 145 mmol/L</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>11</td>
<td>0 - 10 g/L</td>
</tr>
<tr>
<td>WBC #</td>
<td>21.5</td>
<td>5.5 - 17.5 x 10^9/L</td>
</tr>
</tbody>
</table>

HUS, as noted by its name, is merely a syndrome, and therefore, has many known etiologies. Typical (infectious) HUS is caused by bacterial and viral infections such as E. coli 0157:H7, some non-0157 E. coli strains, Shigella dysenteriae 1, Streptococcus pneumoniae, Salmonella typhi, Campylobacter jejuni, and HIV. Non-infectious, atypical HUS may be secondary to (but not limited to) pregnancy and postpartum, organ transplant, glomerulonephritis, systemic lupus erythematosus, or treatment with drug therapies such as tacrolimus (FK506), quinine, and mitomycin. However, it is estimated in the U.S. that 90% of cases of HUS in children are primary infections caused by E. coli 0157:H7 with increasing findings of non-0157:H7 E. coli cases, which may cause higher incidences of HUS in other countries. 13,14

Infection with E. coli 0157:H7 can be acquired from several sources. Enterohemorrhagic E. coli (EHEC) is carried asymptomatically in the intestines of cattle, where Globotriaosylceramide (Gb3) receptors are found throughout the intestinal tract, but cattle lack Gb3 receptors in the vasculature. 15,16 These findings may play a significant role in colonization. However, the reason cattle are asymptomatic carriers of E. coli 0157:H7 is still being studied. During slaughter, the surface of EHEC contaminated meat is ground into and spread throughout the hamburger. Only 50 to 100 viable organisms of E. coli 0157:H7 are required to cause infection. 7 Other food products, besides ground beef, that can be a reservoir for EHEC after contamination with cattle feces include: unpasteurized milk products, apple juice, water, and vegetables. There is seasonality to the shedding of EHEC from cattle, where there is an increased amount of organism shed in the summer months, which correlates with an increase in products with fecal contamination, E. coli 0157:H7 infections, and HUS in the warmer season. 17 The infant in the case study, however, presented with HUS in January.
Although cattle are the major source of _E. coli_ 0157:H7, it has been found in other animals, e.g., sheep, goats, deer, and even a small percentage in cats, dogs, horses, and flies.\(^{18,19}\) _E. coli_ 0157:H7 has been shown to survive ten months or longer in contaminated sources that resulted in human infection.\(^{20}\) Additionally, _E. coli_ 0157:H7 can readily be transmitted from person to person, causing concern to families, nursing homes, daycare facilities, and other crowded living conditions.\(^{21,22}\) After September 11, 2001, _E. coli_ 0157:H7 was considered to be a potential agent of bioterrorism.\(^{23}\) In the case of this thirteen-month old child, no other children or adults with whom he had contact were infected.

**Pathogenesis and pathophysiology**

The pathogenesis of typical HUS begins with the hardiness of _E. coli_ 0157:H7. _E. coli_ 0157:H7 can easily live through freezing at \(-80^\circ\text{C}\) for as long as nine months. Its acid resistance increases survival in acidic foods (conditions that are normally used to preserve food from bacterial contamination) because acidity decreases the nutritional competition with other non-acid-resistant organisms. _E. coli_ 0157:H7 can remain viable in a pH environment less than 2.5.\(^{24}\)

Once _E. coli_ 0157:H7 enters the body orally, it survives the pH of the stomach, and colonizes in the mucosa of the colon. The exact mechanism of _E. coli_ 0157:H7 pathogenicity is under extensive research. A proposed mechanism is that the lipopolysaccharide and STX from the organism are responsible for activating the phosphatidylinositol cascade, increasing mobilized ionized calcium that allows adherence of the organism to the epithelial cells of the colon and promotes lesion formation. Lesions change the permeability of the epithelial cell membrane to water and electrolytes, causing the prodromal diarrhea, and initiate an inflammatory response. Lesions from STX in the typical HUS.

The inflammatory response activates neutrophils that release cytokines, platelet activating factor (PAF), and tissue necrosis factor alpha. These play a role in increasing activated neutrophils (leukocytosis), platelet aggregation,\(^{26}\) and up-regulating Gb03, receptor-specific B subunits on kidney and brain endothelial cells,\(^{12}\) respectively. Close proximity of the injured epithelial cells to microvasculature results in hemorrhagic colitis (HC) and bloody diarrhea in the typical HUS.

STX access to circulation following HC allows for direct and indirect mechanisms of pathogenicity. STX reaches the kidney and brain through the circulatory system on the surface of platelets, neutrophils, and monocytes.\(^{27-29}\) STX bound to platelets induces platelet aggregation directly, and STX prevents apoptosis in neutrophils, resulting in leukocytosis and increased inflammation.\(^{27,30}\)

The proposed primary target of STX from the circulation is the distal convoluted tubular epithelium in children (Gb, receptors may not be present in adults).\(^{31}\) The injured renal epithelial cells also initiate an inflammatory response and release endothelin, tissue plasminogen activator inhibitor-1 (PAI-1), and PAF. Endothelin increases production of PAF, white blood cell activation that results in leukocytosis, and may somewhat increase blood pressure early on in typical HUS. PAI-1 inhibits fibrinolysis of microvascular thrombosis. PAF-activated platelets release thromboxane hence promoting platelet aggregation by vasoconstriction-induced high shear stress. Thromboxane is regulated by prostacyclin released from injured epithelial cells through negative feedback. However, in typical HUS, platelet activation outnumbers production of prostacyclin resulting in thrombosis.\(^{32}\)

Microvascular endothelial cells that have Gb receptors (in children and adults), particularly those of the glomerulus, are extremely sensitive to STX. Injury to the endothelial cells by bound STX is key to the pathogenesis of typical HUS.\(^{7,32}\) STX causes extensive microangiopathic thrombosis, hypoxia, and ischemia in the glomerular endothelial cells, and similar damage can be found in the cerebral endothelial cells of the blood-brain barrier.\(^{33}\) Injury to the endothelial cells is marked by elevated thrombomodulin levels. Lesions from STX involve the glomerular capillaries; thickening of the capillary wall near the glomerulus decreases the glomerular filtration rate.\(^{34}\) Both ischemia and thickened capillaries elevate blood pressure. Progressive damage to the kidneys occurs via hyperfiltration of the functional nephrons that remain after the acute phase.\(^{35}\) Damage to cerebral endothelial cells causes encephalopathy, coma, stroke, and cerebral infarcts.\(^{33}\)

Toxins are freely permeable to the glomerulus, and the large capillary surface area enhances toxin pathogenicity. The concentration of toxin is increased by countercurrent roles of vasculature and tubules in the kidney.\(^{36}\) STX has one enzymatic subunit A and five receptor-specific B subunits. Subunit A invades and kills the renal endothelial cells by endocytosis and inhibiting translation. Subunit B activates neutrophils, releasing proteases and hydrogen peroxide that irreversibly damage renal cells.\(^{37}\) Oxidative substances and fibrin-platelet aggregations occluding the microvasculature fragment red cells, producing schistocytes on the peripheral blood smear.
**Differential diagnosis**

It is critical to differentiate typical HUS from atypical HUS, thrombotic thrombocytopenic purpura (TTP), and disseminated intravascular coagulation (DIC) for both treatment and prognosis (Table 2). For example, typical HUS requires supportive therapy while TTP mandates plasma exchanges and possibly immunosuppressive treatment with glucocorticoids. Misdiagnosis could be life threatening; a significant number of deaths from TTP occur within forty-eight hours of presentation.\(^{38}\)

Atypical causes of HUS are associated with a high mortality rate and recurrences. These causes include complement deficiency involvement, neurological involvement, an inborn error in the metabolism of cobalamin, or a factor H deficiency. Most secondary causes of atypical HUS can be ruled out with patient history. Generally, atypical HUS does not present with leukocytosis or GI prodrome and bloody diarrhea, and is seen more frequently in adults than young children, perhaps simply because adults are likely to have secondary causes.

TTP, a completely separate disease from HUS, is the result of a decreased level of von Willebrand Factor (vWF)-cleaving enzyme (ADAMTS-13) due to a deficiency of ADAMTS-13 or antibodies against it.\(^{39,40}\) The classical presentation of TTP includes fever, central nervous system involvement, and the diagnostic triad seen in classical HUS. However, 25% of typical HUS cases have nervous system involvement and many present with fever.\(^{41}\) Unlike typical HUS, TTP does not generally involve gastrointestinal inflammation, or leukocytosis.\(^{42}\) TTP is usually systemic with severe thrombocytopenia resulting in platelet counts below 20 x 10^9/L, where typical HUS is primarily localized to the kidney with platelet counts between 30 x 10^9/L and 150 x 10^9/L. Risk for TTP is not targeted to a specific age group, like typical HUS. In the future, differentiating TTP from HUS may be easier by measuring the functional ADAMTS-13 level in the plasma.\(^{43}\)

DIC is a systemic activation of both the coagulation cascade and fibrinolysis, and is commonly associated with pregnancy-related complications like atypical HUS. The easiest most reliable way to differentiate DIC is with routine coagulation testing. Prothrombin time (PT), partial thromboplastin time (PTT), and D-dimer levels are abnormal in DIC, but are normal in patients with typical and atypical HUS. However, vitamin K deficiency may prolong the PT, and D-dimer levels may be elevated with a high concentration of fibrin in the microangiopathic thrombus in patients with typical HUS.\(^{44}\)

**LABORATORY FINDINGS AND CLINICAL COURSE**

Upon diagnosis, the thirteen-month-old infant was transferred via ambulance to a major referral childrens hospital. Albumin, total bilirubin, and hemoglobin from the initial laboratory tests (Table 1) were near normal supporting the beginning of the acute phase of HUS. Hemoglobin drops as hemolysis increases during the acute

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### Table 2. Differential diagnosis of typical HUS including patient case study results

<table>
<thead>
<tr>
<th>Laboratory and clinical findings</th>
<th>Patient</th>
<th>Typical HUS</th>
<th>Atypical HUS</th>
<th>TTP</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal kidney tests</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y/N</td>
</tr>
<tr>
<td>Abnormal liver tests</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y/N</td>
</tr>
<tr>
<td>Abnormal PT, PTT, and D-dimer</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>N</td>
<td>Y/N</td>
<td>Y/N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>GI prodrome/bloody diarrhea</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y/N</td>
<td>Y</td>
</tr>
<tr>
<td>Positive Coombs test</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Recurrences</td>
<td>N</td>
<td>N</td>
<td>Y/N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y/N</td>
<td>Y/N</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Y = Yes, N = No
RESEARCH AND REPORTS

phase, and total bilirubin rises as hemoglobin from lysed red cells is broken down, following degradation of the hemoglobin porphyrin ring. A type and screen (T&S) and indirect Coombs were ordered for type-specific supportive transfusions. The indirect Coombs was negative, supporting a non-immune-mediated cause of hemolysis. Clinically, hemolysis is monitored by the hemoglobin level, but LDH and AST tests supplement evaluation of hemolysis. LDH is more specific to hemolysis; however, extremely elevated LDH also reflects tissue necrosis (Table 3).43 Since there is extensive red cell destruction in HUS, most of the haptoglobin is bound to free hemoglobin, giving lower detectable levels of haptoglobin in the serum. Coagulation studies were found to be normal ruling out DIC in the differential diagnosis (Table 3).

Recovery of a small urine sample was obtained from the Foley catheter of the infant for urinalysis (Table 4) which confirmed proteinuric, acidosis, and hemolysis. Red cell casts were not found in this sample, but are commonly found with careful examination in patients with typical HUS.44 The infant began peritoneal dialysis on day 3 (Figures 1, 2, and 3), was given nasogastric tube feedings for nutritional support, and was placed in barrier isolation to prevent transmission of suspected E. coli 0157:H7.

In addition to the urinalysis, stool cultures were ordered for growth of E. coli 0157:H7, Shigella, Yersinia, Salmonella, and Campylobacter. Cultures are done to confirm a causative agent of HUS and for epidemiologic purposes such as tracking of the contaminated food product and disease control. Sorbitol MacConkey agar is generally the medium of choice for isolating the non-sorbitol-fermenting E. coli 0157:H7. Most non-pathogenic E. coli found in the normal flora of the GI tract ferment sorbitol. After successive negative stool cultures for STX-producing organisms, the infant was released from barrier isolation. A negative culture does not rule out a typical HUS diagnosis, and is commonly seen due to the transient shedding of E. coli 0157:H7, culturing too late in the course of the infection, or poor culture sensitivities. The best time to obtain a positive culture for the infectious organism is during the GI prodrome.14

Throughout the infant’s 32-day hospitalization, HUS was monitored with daily basic metabolic panels and cell blood counts. Monitoring hemoglobin, platelets, creatinine, and albumin throughout the acute phase of HUS is indicative of severity and duration of hemolysis, microthrombi, and kidney dysfunction, respectively. An increase in catabolism, during the acute phase of the infection, and the change in distribution of volume to albumin in the serum resulted in hypoalbuminemia, reaching a low of 1.7 mg/dL. Because no platelets were transfused or any other therapy given that would directly affect the platelet count, the rise in platelet count on day-11 marked the initiation of the recovery phase (Figure 2). Day-15 marked the end of the acute phase of the illness with stabilization of the platelet count, hemoglobin, and creatinine (Figures 1-3). After day-15, supportive transfusions were no longer necessary, and on day-20, recovery was confirmed by discontinuation of dialysis with continued stabilization of creatinine levels (Figure 3).

| Table 3. Laboratory evaluation of patient hemolysis and confirmation of typical HUS |
|-----------------|-----------------|------------------|
| Tests          | Patient results | Reference ranges |
| AST            | 176             | 25 - 45 U/L      |
| LDH            | 10240           | 425 - 975 U/L    |
| Lipase         | 160             | 25 - 120 IU/L    |
| PT/INR         | 12.8 sec/1.05   | NA/0.9 - 1.1     |
| PTT            | 32.5            | 24.7 - 33.4 sec  |

| Table 4. Patient urinalysis results on day-2 of hospitalization |
|-----------------|-----------------|------------------|
| Urinalysis and microscopic | Patient results | Reference range |
| Appearance      | cloudy          | clear            |
| Bacteria        | many            | negative         |
| Bilirubin       | negative        | colorless-dark yellow |
| Color           | pink            | negative         |
| Glucose         | negative        | 0-75 mg/dL       |
| Hemoglobin      | 250 Ery/µL      | negative         |
| Ketones         | 5 mg/dL         | 5.0 - 8.5        |
| Nitrite         | negative        | negative         |
| pH              | 5.0             | <75 mg/dL        |
| Protein         | 500 mg/dL       | negative         |
| Red blood cells | <663/HPF        | 0.3 - 0.5         |
| Specific gravity| 1.016           | 1.003 - 1.030    |
| Urobilinogen    | negative        | 0.1 - 1.0 Ehrlich’s units |
| WBC esterase    | 100 LEU/µL     | negative         |
| White blood cells| 66/HPF         | 0.5/HPF          |
As commonly seen in classical typical HUS, the WBC differentials showed a left shift with few myelocytes and metamyelocytes along with leukocytosis and thrombocytopenia. Cell morphology included moderate hypochromic, microcytic red cells with a few schistocytes, nucleated red cells, ovalocytes, polychromasia, and giant platelets. Reticulocyte counts would likely be increased, while the infant attempted to compensate for the anemia with increased red cell production. A bone marrow aspirate is certainly not necessary and would be risky given the presence of thrombocytopenia. However, cellular morphology of the bone marrow would be expected to show normal to increased megakaryocytes (depending on platelet consumption), and erythroid hyperplasia.

As often occurs with HUS, this infant had several complications. On day-2, the infant developed tachycardia, cardiac arrhythmia, and periorbital edema. Cardiac arrhythmias in HUS are due to cardiac ischemia, which can also result in myocardial infarction in typical HUS. On day-4, slight involvement of the pancreas was seen with elevated serum lipase (Table 3). Both lipase and amylase are markers for pancreatitis. Since lipase is not elevated by as many conditions as amylase, it is a more specific indicator for pancreatitis. After the onset of abdominal pain, lipase levels rose within 12 hours and rapidly fell to normal range within a few days. In addition, the infant developed peritonitis when the dialysis catheter was compromised, which was reflected by the increasing creatinine levels at day-9 (Figure 3). A dialysate culture revealed coagulase-negative Staphylococcus sp. and Streptococcus pyogenes, and a dialysate sample revealed a neutrophil count of 1830 x 10^9/L with 75% neutrophils and 23% monocytes with toxic granulation. The infant was treated with vancomycin and serum levels were monitored. On day-25, the infant spiked a fever of 103 °F and was treated with cefotaxime and vancomycin for possible septicemia. Both blood and urine cultures were obtained, and both were found to be negative for growth.

After conclusion of the acute phase, a urinalysis still showed significant proteinuria along with a few granular casts and renal epithelial cells, but the hemoglobin levels and red cell numbers supported the cessation of hemolysis. The infant was discharged on day-32 with resolution of the fever and the acute phase of typical HUS. The infant continues to be closely monitored for long-term prognosis.

**TREATMENT**

The treatment regimen used in the acute phase of typical HUS is primarily supportive and may include medication to control blood pressure, continuous peritoneal dialysis or hemodialysis, packed red cell transfusions, fluid restriction, diuretics, and very careful maintenance of electrolytes. In rare cases, a kidney transplant may be required. Untreated typical HUS will lead to coma, cerebral infarcts, pancreatic insufficiency, and death. However,
rigorous management of treatment has decreased the mortality rate of typical HUS from 50% to about 5% to 10% throughout the last five decades. \(^{47,48}\)

There are important contraindications to certain treatments when typical HUS is diagnosed. Platelets are generally not transfused, because they may cause more severe microangiopathic thrombosis. Antibiotics are also not suggested, because killing \(E. coli\) 0157:H7 may rupture the organism’s cell wall, thereby increasing the amount of toxin released, and contributing to the severity of the acute phase. \(^{49}\) If complications occur, however, during typical HUS, such as peritonitis or sepsis, antibiotics may be necessary. Anti-motility drugs for diarrhea are not advised, because they may increase the risk of typical HUS by extending the exposure time of STX to the patient’s cells. \(^{50}\)

Long-term treatment for patients requires monitoring of blood pressure, creatinine, and urinalysis. \(^{51}\) Patients exhibiting hypertension and proteinuria are treated to lower the blood pressure with an angiotensinogen-converting enzyme (ACE) inhibitor (blocking the production of renin and consequently, aldosterone) to reduce further damage to the glomerulus, reduce proteinuria, and delay or prevent future chronic renal failure. \(^{52}\) Two years of post-HUS laboratory testing on this infant confirmed hypertension and proteinuria. After beginning treatment with an ACE inhibitor, the infant showed a decrease in blood pressure and proteinuria.

**PROGNOSIS**

Although prognosis of HUS remains variable, a recent meta-analysis produced significant data. It is estimated that 58% of HUS patients fully recover, 5% die within the acute phase, 11% develop chronic renal failure or die within four years of the acute phase, and 17% experience long-term residual effects such as hypertension, proteinuria, and a decreased glomerular filtration rate. There is often a period of perceived renal recovery before the onset of long-term residual effects. Surprisingly, even some patients who presented with mild HUS (without dialysis or abnormal urine output) developed renal sequelae. \(^{47}\) Renal sequelae may result in renal failure more than twenty years after the acute phase of HUS. \(^{53}\) Thus, it is extremely important that the renal function of any child with a history of HUS be followed for life.

The best practical indicator of prognosis is the duration of oliguria or anuria and consequently, the need for dialysis during the acute phase of HUS. \(^{54}\) The most sensitive indicator is a kidney biopsy, which shows the degree of renal cortical necrosis. This procedure is impractical and contraindicated in the acute phase, but it is used later to assess long-term prognosis. \(^{53,55}\) Other factors that may support poor long-term prognosis include: elevated white cell count \(>20 \times 10^9/L\), neural involvement, post-HUS proteinuria, and hypertension during the acute phase. \(^{35,56-58}\) In the case history of the thirteen month old infant, the following poor prognostic indicators were found: oliguria/anuria \(>10\) days, leukocytosis \(>20 \times 10^9/L\), post-HUS proteinuria, and hypertension at onset of HUS.

**PREVENTION**

There are three important levels of prevention when dealing with \(E. coli\) 0157:H7. On the public level, proper handwashing and safe preparation of food remain the best practices for the prevention of typical HUS. However, too much responsibility has been placed on the public. \(^{59}\) The state and federal government play critical roles in prevention. They are responsible for keeping the food supply safe and ensuring quality assurance by mandating effective screening of food products for \(E. coli\) 0157:H7 and other foodborne pathogens. In the past, only a few states required such screening. With the new rapid STX detection methods available, surveillance of \(E. coli\) 0157:H7 in the beef industry is improving. \(^{60}\) The Food and Drug Administration has approved the irradiation of ground beef, which can kill at least 90% of \(E. coli\) 0157:H7 contaminants. \(^{54}\) A law mandating irradiation of other potentially contaminated food products was approved by the United States Department of Agriculture and is being implemented. Cases involving contaminated water sources have also called attention to enforcing regulations for protecting small water systems that in the past received little attention. \(^{61}\)

**RESEARCH AND THE FUTURE**

Current research is aimed at faster and more reliable testing of STX as well as countering the action of STX produced by any organism. The conventional method involving the culture of stool on MacConkey-Sorbitol agar for \(E. coli\) 0157:H7 is only 40% sensitive and takes up to three days to complete. \(^{62}\) Further complicating the diagnosis of typical HUS is the usual delay of symptoms for one to two weeks after infection, leaving few organisms in the stool for detection. In addition, more than 100 other shiga toxin-producing \(E. coli\) (STEC) serotypes are left undetectable by many clinical laboratories because they ferment sorbitol, like normal intestinal strains of \(E. coli\). \(^{63}\)

Improved technology is strongly needed to enhance the sensitivity of culture for \(E. coli\) 0157:H7 in HUS patients.
In 1996, research showed a success rate of 90% for *E. coli* 0157:H7 if an immunomagnetic separation (IMS) technique was used to isolate the 0157 antigen (using beads with the corresponding antibody) after use of a pre-enrichment culture medium. This method proved to have many advantages over new PCR methods for detecting *E. coli* 0157:H7 including at least a 100-fold increase in sensitivity, and less complex testing. In 1999, the United States Department of Agriculture began implementing IMS to improve the screening of ground beef. To date, many clinical laboratories have not adopted the improved methods for routine screening of *E. coli* 0157:H7 in children suspected of HUS. If a clinical laboratory is unable to implement new technology for detecting *E. coli* 0157:H7 and/or STX, the specimen should be referred to an appropriate reference laboratory.

Alternative rapid tests for non-0157 STEC are directed toward the detection of STX produced instead of the organism. One method uses an EIA technology that requires approximately 18 hours to perform (this includes a 16-hour incubation period for increased sensitivity), while another method is a simple toxin detection test that only takes three hours to perform. Both methods may significantly shorten the time for diagnosis. In addition, other improved methods include PCR followed by pulse-field gel electrophoresis, impedance technology, and enzyme-linked immunoassays.

Vaccination is another potential area of interest. Healthy persons develop an antibody to the antigenic subunit B of STX after exposure. When mice were immunized with the B subunit of STX, they were able to produce antibodies that neutralized the potent effects of STX. Monkeys have also been successfully immunized and protected by an STX vaccine when challenged with a lethal dosage. Immunizations may be administered for both humans and cattle in order to reduce *E. coli* 0157:H7 infections and incidence of typical HUS.

Treatment of patients with monoclonal antibodies, currently in clinical trials, may neutralize the STX in patients presenting with early diagnosis of typical HUS. The monoclonal STX antibody binds STX in the intestine and may render the toxin less able gain access to the circulation. Hence, STX is less likely to affect the kidney or other organs. One remaining concern with this treatment is the remaining lipopolysaccharide (LPS) of the bacteria that also plays an antigenic role in typical HUS, perhaps warranting the need for treatment with another monoclonal specific antibody or a polyvalent antibody to both STX and LPS.

**CONCLUSION**

As in the case of this thirteen-month-old infant with HUS, it is estimated that *E. coli* 0157:H7 causes 73,000 of the 76 million food-borne illnesses each year. Most food-borne illnesses in the U.S. are self-limiting, but those that cause HUS may lead to death or life-long consequences such as renal sequelae and gastrointestinal complications. Progress has been made in the elimination of food-borne causes of typical HUS via irradiation of food products and improved quality assurance and control in the food industry. Current research is focused on vaccination, improved diagnostic testing methods, and intervening treatments with early diagnosis. “So HUSH all you children and don’t you cry, together we will beat the bug they call *E. coli***.”

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Clinical Laboratory Science Announces 2004 Distinguished Author Award Recipients

Recipients of the *Clinical Laboratory Science* (Clin Lab Sci) Distinguished Author Awards are chosen by Clin Lab Sci readers and editorial board members. Nominations are based upon originality, quality of writing, and relevance and value to the clinical laboratory science profession. The Editorial Board of *Clin Lab Sci* is pleased to announce the following recipients of the 2004 Distinguished Author Awards:

Reports and Reviews

Vicky A LeGrys, Katherine Hartmann, and Joan R Walsh for their article *The Clinical Consequences and Diagnosis of Hypothyroidism* published in the Fall 2004 issue of *Clinical Laboratory Science*.

Research

Isaac D Montoya for his article *Topography as a Contextual Variable in Infectious Disease Transmission* published in the Spring 2004 issue of *Clinical Laboratory Science*.

Focus Section

Louann W Lawrence for her article refractory *Anemia and the Myelodysplastic Syndromes* published in the Summer 2004 issue of *Clinical Laboratory Science*. 
2003 Workforce Survey of Hospital Clinical Laboratories in New Jersey

ELAINE M KEOHANE, MARY ELLEN SCHAAD, KAREN FEENEY

ABBREVIATIONS: CT = cytotechnologist; HLT = histotechnologist; HT = histotechnician; MLT = medical laboratory technician; MT = medical technologist.

INDEX TERMS: job opportunities; workforce.


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The authors are also members of the Coalition for New Jersey Clinical Laboratory Personnel.

The clinical laboratory personnel shortage has reached significant proportions in many areas of the country and there is growing concern about its impact on the accessibility and quality of clinical laboratory services. For the years 2002–2012, the U.S. Bureau of Labor Statistics projected a need for 138,000 new clinical laboratory technologists and technicians, or approximately 13,800 per year, due to growth and attrition from the field.1 On the other hand, in 2002, there were only 3,548 clinical laboratory technician/medical laboratory technician (CLT/MLT) and clinical laboratory scientist/medical technologist (CLS/MT) graduates in the U.S.2 If the current imbalance between vacancies and graduates continues, the national shortage of clinical laboratory personnel may grow by more than 10,000 laboratorians per year. In surveys conducted by the American Society for Clinical Pathology (ASCP), vacancy rates in 2000 for medical technologists (MTs) and medical laboratory technicians (MLTs) were 11.1% and 14.3% nationally and 14.9% and 24.5% in the northeast; in 2002 those rates showed a decrease to 7% and 8.6% nationally and 8.3% and 3.5% in the northeast. Although the vacancy rates in the latter study decreased to single digits, the vacancies are nevertheless noteworthy in terms of the actual number of vacant positions, taking into consideration a national workforce estimated at 297,000 clinical laboratory technologists and technicians.1

A Coalition for New Jersey Clinical Laboratory Personnel was formed in April 2002 to study the extent of and address a perceived shortage of clinical laboratory personnel in New Jersey. This coalition consists of twenty-eight members representing hospital clinical laboratory administrators, supervisors, and educators; hospital human resources directors; and representatives from the New Jersey Society for Clinical Laboratory Science, New Jersey Clinical Laboratory Management Association, New Jersey Hospital Association, the New Jersey State Department of Health and Senior Services, and New Jersey Medicaid. One of the goals of the coalition is to document and disseminate data on the supply of and demand for clinical laboratory professionals in the state. An unpublished study conducted by the New Jersey Society for Clinical Laboratory Science showed a 48.5% decrease in MLT and MT graduates since 1998, with only 26 MTs and 21 MLTs graduating in 2003 in the entire state. In addition, during that same time, the state experienced the closure of one MT and three MLT programs. There are no histotechnologist (HTL) programs, and only one histotechnician (HT) program in the state, but that program recently went
into inactive status. There is also only one cytotechnologist (CT) program. In 2003, these programs produced only seven HT and five CT graduates.

Although there was some anecdotal information from New Jersey laboratory managers about difficulties in hiring qualified laboratory practitioners, there was insufficient data on vacancies and shortages for these practitioners in the state’s workforce. Therefore, the Coalition conducted a survey of hospital clinical laboratory managers to determine the extent of the clinical laboratory personnel shortage in NJ, and to begin a data collection process to project workforce needs into the future.

**METHODS**

In January 2003, a one-page survey was mailed to the clinical laboratory managers of the 95 hospitals in NJ. Surveys were coded for tracking purposes. A second survey was sent in March 2003 to the non-responders, followed by phone contact.

The survey requested data on county, hospital size, total number of billable tests, total current budgeted FTEs, the number and age of clinical laboratory employees in six categories, the number of current vacancies, and the average time it took to fill vacancies. The six personnel categories included MT staff, MT supervisor, MLT, HTL, HT, and CT. In addition, managers were asked to indicate if they had difficulties hiring or recruiting for a particular position, department, or shift, and if they had incentives in place to hire laboratory personnel. All survey responses were received between February and April 2003, and were reviewed, tabulated, and summarized.

**RESULTS**

A total of 55 surveys were received for a response rate of 57.9%. Forty-nine (51.6%) of the surveys contained data that were usable in the analysis, and represented data from hospitals in fifteen of the twenty-one NJ counties (Table 1). A majority of the surveys (31) were received from the Northern NJ counties.

Forty-seven percent of the usable responses were from hospitals with greater than 300 beds, while 53% had less than 300 beds. A total of 33,094,905 annual billable laboratory tests were reported by 39 hospitals, ranging from 97,800 to 3,822,755 per hospital. A total of 2,697 total budgeted FTEs were reported by 49 hospitals.

Figure 1 depicts the breakdown of all categories of NJ clinical laboratory personnel by age. A breakdown of personnel by category and age is depicted in Figure 2 and Table 2.

The largest category reported was MT staff with 1,455 employees in 49 hospitals. In the MT staff category, 50.8% of the employees were over 45 years, and 14% were over 55 years, while 61.5% of the MT supervisors were over 45 years, with 21.6% over 55 years. The MLT population was somewhat younger with only 38.3% over 45 years. In the MLT, HTL, HT, and CT categories, 10% of the employees were over 55 years.

Table 3 lists the number of vacancies by category and region. The highest number occurred in the MT category with 49 full time and 52 part time vacancies among the

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**Table 1.** Usable surveys by New Jersey County. Numbers represent surveys received by region and by county

<table>
<thead>
<tr>
<th>Region</th>
<th>North - 31</th>
<th>Central - 9</th>
<th>South - 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergen</td>
<td>5</td>
<td>Hunterdon - 0</td>
<td>Atlantic - 3</td>
</tr>
<tr>
<td>Essex</td>
<td>9</td>
<td>Mercer - 3</td>
<td>Burlington - 1</td>
</tr>
<tr>
<td>Hudson</td>
<td>6</td>
<td>Middlesex - 1</td>
<td>Camden - 3</td>
</tr>
<tr>
<td>Morris</td>
<td>0</td>
<td>Monmouth - 5</td>
<td>Cape May - 0</td>
</tr>
<tr>
<td>Passaic</td>
<td>6</td>
<td>Somerset - 0</td>
<td>Cumberland - 0</td>
</tr>
<tr>
<td>Sussex</td>
<td>1</td>
<td></td>
<td>Gloucester - 1</td>
</tr>
<tr>
<td>Union</td>
<td>2</td>
<td></td>
<td>Ocean - 1</td>
</tr>
<tr>
<td>Warren</td>
<td>2</td>
<td></td>
<td>Salem - 0</td>
</tr>
</tbody>
</table>

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**Figure 1.** Percent distribution of all categories of NJ laboratory personnel by age (n = 2,004). Note that 49.1% are older than 45 years, and 13.8% are older than 55 years
49 hospitals. In the MT supervisor category, there were 11 full time and 4 part time vacancies, and in the MLT category, there were 6 full time and 20 part time vacancies. There were seven vacancies for full time HT and one vacancy for a full time CT.

Table 2. Breakdown of clinical laboratory personnel by category and age

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>MT staff</th>
<th>MT Supervisor</th>
<th>MLT</th>
<th>HTL</th>
<th>HT</th>
<th>CT</th>
</tr>
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<tbody>
<tr>
<td>Current total # (n = 49 hospitals)</td>
<td>2331</td>
<td>1455</td>
<td>251</td>
<td>428</td>
<td>34</td>
<td>116</td>
<td>47</td>
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<tr>
<td>Current total # reported by age (n = 42 hospitals)</td>
<td>2004</td>
<td>1247</td>
<td>213</td>
<td>381</td>
<td>28</td>
<td>98</td>
<td>37</td>
</tr>
<tr>
<td>Under 35 yrs</td>
<td>315</td>
<td>177</td>
<td>15</td>
<td>90</td>
<td>4</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>15.7%</td>
<td>14.2%</td>
<td>7.0%</td>
<td>23.6%</td>
<td>14.3%</td>
<td>21.4%</td>
<td>21.6%</td>
</tr>
<tr>
<td>36 – 45 yrs</td>
<td>706</td>
<td>436</td>
<td>67</td>
<td>145</td>
<td>9</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>35.2%</td>
<td>35.0%</td>
<td>31.5%</td>
<td>38.1%</td>
<td>32.1%</td>
<td>36.8%</td>
<td>35.2%</td>
</tr>
<tr>
<td>46 – 55 yrs</td>
<td>708</td>
<td>460</td>
<td>85</td>
<td>108</td>
<td>12</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>35.3%</td>
<td>36.8%</td>
<td>39.9%</td>
<td>28.3%</td>
<td>42.9%</td>
<td>31.6%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Over 55 yrs</td>
<td>275</td>
<td>174</td>
<td>46</td>
<td>38</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>13.8%</td>
<td>14.0%</td>
<td>21.6%</td>
<td>10.0%</td>
<td>10.7%</td>
<td>10.2%</td>
<td>10.8%</td>
</tr>
<tr>
<td>45 or younger</td>
<td>1021</td>
<td>613</td>
<td>82</td>
<td>235</td>
<td>13</td>
<td>57</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>50.9%</td>
<td>49.2%</td>
<td>38.5%</td>
<td>61.7%</td>
<td>46.4%</td>
<td>58.2%</td>
<td>56.8%</td>
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<tr>
<td>46 or older</td>
<td>983</td>
<td>634</td>
<td>131</td>
<td>146</td>
<td>15</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>49.1%</td>
<td>50.8%</td>
<td>61.5%</td>
<td>38.3%</td>
<td>53.6%</td>
<td>41.8%</td>
<td>43.2%</td>
</tr>
</tbody>
</table>

Figure 2. Percent distribution of categories of laboratory personnel by age
RESEARCH AND REPORTS

Table 3 summarizes the responses for the average time to fill vacancies in the various categories. The time ranged from an average of 10.6 and 11 weeks for MLTs and MTs, respectively, to 104 weeks for HTs.

A majority of laboratory managers reported difficulties in hiring generalist MTs (71.4%), as well as night shift (46.9%), evening shift (28.6%), HT (24.5%), and blood bank (20.4%) positions. Other positions in which difficulties in hiring were reported included part time, weekend, CT, and general supervisory positions. Figure 3 presents a summary of the data. The miscellaneous category includes one to two responses each for microbiology technologist, phlebotomist, evening supervisor, blood bank supervisor, and histology supervisor.

Laboratory managers reported using the following incentives for hiring laboratory personnel: 42.9% of laboratory managers reported no incentives, 30.6% had shift differentials, 26.5% had tuition reimbursement, 24.5% had a sign-on bonus, and 20.4% made market adjustments in salaries. The incentives are summarized in Figure 4. In addition, some laboratory managers had comments related to their hiring difficulties. These are summarized in Table 5.

DISCUSSION

The data reflect responses from over half of the hospital clinical laboratories in NJ. The largest number of surveys being received from the northern region of the state reflects the larger number of hospitals in that region. The responding hospitals were almost evenly divided between those that are greater than 300 beds and those that are less than 300 beds.

A total of 33,094,905 annual billable tests were reported by 39 hospitals. Projecting that figure across 95 hospitals at an average cost of $14 per test, hospital laboratories represent a billion dollar industry in the state.

Table 3. Number of vacancies by category and region (n = 49 hospitals)

<table>
<thead>
<tr>
<th></th>
<th>MT Staff</th>
<th></th>
<th>MT Sup</th>
<th></th>
<th>MLT</th>
<th></th>
<th>HTL</th>
<th></th>
<th>HT</th>
<th></th>
<th>CT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>FT</td>
<td>49</td>
<td>26</td>
<td>18</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PT</td>
<td>52</td>
<td>36</td>
<td>12</td>
<td>4</td>
<td>20</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FT = full time; PT = part time; Sup = supervisor; Tot = total number vacancies; N = vacancies in northern NJ; C = vacancies in central NJ; S = vacancies in southern NJ

Table 4 summarizes the responses for the average time to fill vacancies in the various categories. The time ranged from an average of 10.6 and 11 weeks for MLTs and MTs, respectively, to 104 weeks for HTs.

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<th></th>
<th>MLT</th>
<th></th>
<th>HTL</th>
<th></th>
<th>HT</th>
<th></th>
<th>CT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
<td>Tot</td>
<td>N</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>FT</td>
<td>49</td>
<td>26</td>
<td>18</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>5</td>
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</tr>
<tr>
<td>PT</td>
<td>52</td>
<td>36</td>
<td>12</td>
<td>4</td>
<td>20</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FT = full time; PT = part time; Sup = supervisor; Tot = total number vacancies; N = vacancies in northern NJ; C = vacancies in central NJ; S = vacancies in southern NJ

Figure 3. Percentage of laboratory managers reporting hiring difficulties by category (n = 49)
Nearly half of the practitioners employed in the responding NJ hospital clinical laboratories are over the age of 45, which is only slightly lower than the median age of 47 years reported in 2002 for clinical laboratory practitioners nationwide. In NJ, 13.8% of the clinical laboratory professionals in the 49 responding hospitals were over the age of 55; projecting that number over the 95 hospitals in the State, it is likely that over 500 laboratorians in the current workforce will be over 65 within the next 10 years.

There were a total number of 2,697 budgeted FTEs in 49 hospitals, with 2,331 current clinical laboratory employees. Using these figures, an estimate of the overall vacancy rate for hospital laboratory personnel is 13.6%.

During the 3-month survey period, there were a total of 49 full time and 52 part time vacancies for staff MTs, and 11 full time and 4 part time vacancies for supervisory MTs in the 49 responding hospitals. Projecting those figures to the 95 hospitals in the state, the number of actual vacancies could be as high as 116 full time and 109 part time MT positions. Given that only 26 MTs graduated in 2003, and that not all of that cohort will choose to work in hospital laboratories, there was a significant shortfall of MTs with approximately eight vacant MT positions per MT graduate. Over 70% of the laboratory managers reported difficulty in filling vacant MT generalist positions, while difficulty in filling night, evening, and blood bank positions were reported by 46.9%, 28.6%, and 20.4% of the managers, respectively. As indicated in a comment in Table 5, an unknown, but probably significant, number of MTs work more than one job to supplement their salaries. If this did not occur, (assuming the second job is in another hospital laboratory) the shortage would be even greater.

During this same period, there were 4 full time and 20 part time MLT vacancies. Again, projecting these figures to the 95 hospitals in the state, the amount of actual vacancies could be as high as 8 full time and 39 part time positions. Given that only 21 MLTs graduated in 2003, and that not

### Table 4. Average time to fill vacancies

<table>
<thead>
<tr>
<th>Average number of weeks</th>
<th>MT Staff</th>
<th>MT Sup*</th>
<th>MLT</th>
<th>HTL</th>
<th>HT</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of weeks</td>
<td>11.0</td>
<td>14.8</td>
<td>10.6</td>
<td>22.6</td>
<td>25.6</td>
<td>25.4</td>
</tr>
<tr>
<td>Range in weeks</td>
<td>1 to 52</td>
<td>2 to &gt;52</td>
<td>2 to 52</td>
<td>8 to &gt;52</td>
<td>8 to 104</td>
<td>12 to 78</td>
</tr>
<tr>
<td>Number of hospitals</td>
<td>n = 39</td>
<td>n = 32</td>
<td>n = 27</td>
<td>n = 8</td>
<td>n = 21</td>
<td>n = 10</td>
</tr>
</tbody>
</table>

* Sup = supervisor

### Figure 4. Percentage of hospitals offering hiring incentives for laboratory personnel (n = 49)
Table 5. Comments from laboratory managers about hiring difficulties

“Most employees are working more than one job to earn a living. This makes it very difficult to staff the lab.”

“Shift differential increases are desperately needed but being delayed at least 6 months due to reimbursement problems.”

“Much difficulty in recruiting supervisors due to lack of qualified outside applicants and lack of interest in qualified in-house staff due to salary compression with increased responsibilities.”

“We gave up trying to fill a part time MLT day job and converted it to a lab aide.”

“Blood bank position vacant since 3/02; histologist position vacant since 8/02.”

“Could not find a CT, so closed cytology.”

“Unable to find qualified candidate for blood bank supervisor. Three blood bank techs went to industry. Histotech asking salary was above our approved range.”

“We are lucky enough to have a MT school; CTs and HTs are extremely hard to find.”

all of that cohort will work in hospital laboratories, there was a shortage of MLTs to fill the open part time positions. Some laboratory managers indicated that they would hire a MLT to fill a vacant MT position if a qualified MT was not available, prompting them to reevaluate their staffing mix of MLTs vs. MTs. If this staffing shift should occur, there will likely be more vacant MLT positions, thus exacerbating the MLT shortage.

Although there were only two vacancies for CTs, there is only one program in the state producing five to eight graduates per year, many of whom are employed, often at higher salaries, by reference laboratories. This situation creates difficulties in filling vacancies at some hospitals, requiring as long as 78 weeks to fill a CT vacancy (Table 4). In fact, one hospital reported closing their cytology department since they were unable to fill a vacant CT position (See Table 5).

There are no HTL programs in the state, and only the one HT Program in the southern region of the state. That one HT Program, however, recently went inactive. It was producing five to ten graduates per year; however, the graduates traditionally preferred employment in their home region, resulting in unfilled positions in the northern and central regions of the state. Nearly 25% of the laboratory managers reported difficulty in filling HT positions, with up to 104 weeks required to fill a vacant position (Table 4). With no active HT or HTL programs in the state, the personnel shortages in histology will become even greater.

The U.S. Bureau of Labor Statistics projected that between 2002–2012 the clinical laboratory field would experience a combination of growth and attrition requiring 13,800 new clinical laboratory technologists and technicians per year. Although the simplification and automation of tests will result in some loss of positions, a net increase in need for new practitioners was predicted due to the population growth, an increase in the elderly population, and the introduction of new types of tests that will spur the utilization of more laboratory services.

The general staffing pattern was 3.4 MTs to 1 MLT in NJ hospital laboratories. Utilization of MLTs to fill some of the vacant MT positions may help to alleviate the shortage and spur an increase in enrollments in MLT Programs.

Supply and demand in the state’s clinical laboratory workforce will continue to be monitored by surveying the educational programs and laboratory managers every two years, and developing a prediction model for future needs. It is also recommended to continue to study and address working conditions, incentives, and salaries to make the clinical laboratory profession more attractive and competitive with other fields that require comparable education, and to begin to study the impact of the shortage on the delivery and accessibility of quality laboratory services. This information is critical for strategic planning for both laboratory managers and educators.

The Coalition launched a Website in May 2003 (www.labscience.org) to inform potential students and science teachers about the clinical laboratory professions. The Coalition will
continue to address the shortage through efforts to promote awareness of the professions to potential students and the public, to assist in recruitment of students into the state’s educational programs, to encourage hospitals, colleges, and universities to maintain their educational programs, and to share information about successful retention programs to keep our highly skilled professionals in hospital settings.

Nationwide, and statewide, clinical laboratory practitioners are not being produced in sufficient quantity to meet the current and future demand. Nationwide, it has been estimated that the shortage is growing by over 10,000 laboratorians per year. In NJ, even if the five existing MT programs operate at full capacity (an estimated 52 students per year), there will still be insufficient graduates to meet the current and the projected demand for MTs in the future. In addition, due to the location of a large number of pharmaceutical companies and commercial laboratories in NJ, the state’s hospitals must compete for skilled laboratory personnel with those industries that recruit professionals out of the hospital setting with higher wages and/or better working conditions, e.g., no weekends, holidays, etc. MT programs either need to expand or new programs need to be established, and perhaps the hospital laboratory industry in the state should re-examine the utilization of MLTs vs. MTs in the workplace.

REFERENCES
FOCUS: PSYCHOSTIMULANTS

Mechanism of Action and Therapeutic Uses of Psychostimulants

KEVIN F FOLEY

ABBREVIATIONS: ADHD = attention-deficit hyperactivity disorder; CNS = central nervous system; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxyamphetamine; NC = not controlled; OTC = over the counter.

INDEX TERMS: psychostimulants.


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Focus Continuing Education Credits: see pages 124 to 126 for learning objectives, test questions, and application form.

The name stimulant can be given to any drug that increases the rate of a physiologic function. The term psychostimulant is more specific, referring to compounds that have direct neurological effects, typically: heightened alertness, increased energy, appetite suppression, and sometimes euphoria. Use of psychostimulants is widespread and occurs in both recreational and clinical settings. Therapeutic monitoring and screening for use and abuse of these drugs is common in clinical laboratories. Understanding the physiological effects and uses of stimulants will be of value to clinical and forensic laboratory scientists.

EXAMPLES OF PSYCHOSTIMULANTS

Table 1 lists several psychostimulants that are commonly prescribed, have widespread illicit use, or are well-known by the general public. Table 1 also identifies the control schedule of each compound. Many psychostimulants are listed as controlled substances under the Controlled Substances Act, Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970. Schedules I through IV are assigned to compounds based on their abuse potential and their medical utility. Schedules II through V contain drugs which have known medical uses whereas Schedule I compounds have no current, sanctioned medical use.

THERAPEUTIC USES OF PSYCHOSTIMULANTS

Amphetamine has long been known to be a mental stimulant. Because of its psychostimulant properties, amphetamine has been used successfully by U.S. fighter pilots because it enhances cockpit performance by reducing the effects of fatigue. Since amphetamine heightens alertness it has found use in the treatment of narcolepsy and in attention-deficit hyperactivity disorder. The drug Adderall® for example, is used widely in cases of ADHD and is merely a mixture of amphetamine's stereoisomers: dextro- and levoamphetamine.

Amphetamine also has appetite suppressant effects and thus is used in the treatment of obesity, although it is not FDA approved for this use. Although amphetamine can be considered the prototype psychostimulant, many psychostimulants have been identified or created that resemble amphetamine in their chemical structures. These compounds vary in their effects and utility as well as their abuse potential. Methamphetamine, like amphetamine has a history of illicit use and is known to be considerably more potent in vivo than the unmethylated amphetamine. Methamphetamine, like amphetamine, has been used with some success in ADHD patients. It is also approved for use in treating obesity.

Pseudoephedrine, the name given to the 1R2R enantiomer, and ephedrine, the 1R2S enantiomer, are common medications used primarily as nasal decongestants. Unlike amphetamine and methamphetamine they have little abuse potential allowing them to be obtained OTC. Ephedrine is also approved for treatment of bronchospasm, enuresis, hypotension, and narcolepsy.

Some psychostimulants have been found to have uses that are not typical for other drugs in the same class. Although bupropion is a phenylalkylamine, the structural dissimilarity with amphetamine is significant enough to produce considerably different physiologic effects. Bupropion is an
example of a weak psychostimulant that has found use as an antidepressant (Wellbutrin®) and is also marketed as a smoking cessation aid (Zyban®). Although still a mild psychostimulant, the behavioral effects of bupropion are reported to be unlike amphetamine when used in humans. The abuse potential is also considerably less than with amphetamine.7 Off-label uses of bupropion include treatment of ADHD, diabetic neuropathy, and neuropathic pain.8 Another clinically-useful β-ketone psychostimulant is diethylpropion. Diethylpropion is currently used only in the treatment of obesity. This psychostimulant is commonly prescribed as an appetite suppressant under the name Tenuate®.

Methcathinone is a Schedule I psychostimulant that is easily synthesized from ephedrine. Methcathinone is the N-methylated version of cathinone, a natural psychostimulant obtained from the Catha edulis plant and known as “qat” or “khar”.9 Methcathinone has no sanctioned medical use and is only used recreationally. Its effects are those of a classic psychostimulant, causing ‘flying euphoria’ followed by a five-to-eight hour period of feeling tough, invincible with increased libido, and desire to be physical.8,9

The next two compounds listed in Table 1 are also Schedule I compounds. 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) are popular recreational drugs known respectively as ‘the love (or hug) drug’ and ‘ecstasy’. MDMA use is growing rapidly and in some European countries is the second most frequently used illegal drug after marijuana.10 Interestingly, both MDMA and MDA were used therapeutically before being classified Schedule I in 1985. These compounds were used by patients in constructive psychotherapy sessions.8 MDMA has received much negative attention in recent years as claims of its neurotoxicity mounted. However data concerning the neurotoxicity of MDMA have been inconsistent and one major study was even retracted after it was found that the toxicity in the study was brought about by methamphetamine rather than MDMA.11,12 The widespread use and popularity of MDMA coupled with the lack of definitive reports on neurotoxicity have encouraged some to reconsider the utility of this drug.13-15 Because of MDMA’s unique effects, a new study investigating the therapeutic use of MDMA has recently earned IRB and FDA approval. This research will assess the use of MDMA in the treatment of post-traumatic stress disorder.16,17

Perhaps the most well-known psychostimulant after amphetamine is methylphenidate, made popular under the trade name Ritalin®. Methylphenidate has pharmacological effects similar

### Table 1. Some common psychostimulants

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trade or common name</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>Dextedrine®, Adderall®</td>
<td>C-II</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>Desoxyn®, Methampex® ‘speed’, ‘crystal meth’</td>
<td>C-II</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>Herbal sources: ma huang, ephedra, ephedra sinica, epitonin, Pretz-D®</td>
<td>OTC</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Wellbutrin®, Zyban®, Buproban™</td>
<td>NC</td>
</tr>
<tr>
<td>Diethylpropion</td>
<td>Tenuate®, Dipro™ Durad™ Radtue™</td>
<td>C-IV</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>‘cat’</td>
<td>C-I</td>
</tr>
<tr>
<td>3,4-Methylenedioxyamphetamine</td>
<td>MDA, ‘love drug’</td>
<td>C-I</td>
</tr>
<tr>
<td>3,4-Methylenedioxymethamphetamine</td>
<td>MDMA, ecstasy, XTC</td>
<td>C-I</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>Concerta™, Metadate®, Methylin™, Ritalin®</td>
<td>C-II</td>
</tr>
<tr>
<td>Phenetermine</td>
<td>Adipex-P®, Fastin®, Obenix®, Phentamine®, Supramine™</td>
<td>C-IV</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>Pondimin® (discontinued, removed from the U.S. market)</td>
<td>C-IV</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Methyl benzoyllecgonine, coke, crack</td>
<td>C-II</td>
</tr>
</tbody>
</table>

C-I = Schedule I: Compounds with high potential for abuse having no currently accepted medical use in treatment in the United States.
C-II = Schedule II: Compounds with high potential for abuse having currently accepted medical use(s) in treatment in the United States or a currently accepted medical use with severe restrictions.
C-IV = Schedule IV: Compounds having a low potential for abuse relative to drugs scheduled as C-III.
NC = not controlled (unscheduled).
OTC = over the counter.
to amphetamine; however, its abuse potential is somewhat lower, although there are many conflicting reports regarding methylphenidate abuse potential (see reference 18 for review). Methylphenidate has become the drug of choice for treating ADHD but has also found off-label use as an antidepressant.5

Two other drugs worth mentioning as psychostimulants are phentermine and fenfluramine. Both of these drugs have been used as appetite suppressants and both have amphetamine-like sympathomimetic effects. Fenfluramine contains a chiral center and fenfluramine is the name given to the racemic mixture of D- and L-isomers. The D-isomer (dexfenfluramine) was purportedly responsible for the anorectic actions of fenfluramine and was also associated with fewer side effects than the racemic fenfluramine. Dexfenfluramine was marketed as Redux®.19 By 1997 both fenfluramine and dexfenfluramine were removed from the U.S. market at the request of the FDA when cases of cardiac valvulopathy were reported.5,20,21 Fenfluramine is perhaps best known by its off-label use with phentermine, known as “Fen-phen”. Fen-phen was widely used for the long-term management of obesity. The fenfluramine-phentermine combination was also associated with valvulopathies and a bulletin was issued by the FDA to cease off-label use of the Fen-Phen combination.5,21 Of the two drugs, only phentermine is still used as an appetite suppressant although tolerance to the anorexiant effects of phentermine usually develops a few weeks after starting therapy.

When considering the clinically-used drugs listed in Table 1, cocaine is the only one that is not used for its psychostimulant effects. Cocaine is used clinically only as a local anesthetic, usually in mucosal or ophthalmic procedures.

MECHANISM OF ACTION
It is easy to see the resemblance between the chemical structures of common psychostimulants and endogenous monoamine neurotransmitters (Figure 1). The prototype psychostimulant amphetamine closely resembles the catecholamine neurotransmitters norepinephrine, epinephrine, and dopamine. Since many psychostimulants share the features of a phenyl ring, a nitrogen group, and carbon side chains of varying lengths, many stimulants fall into the category of phenylalkylamines. With the possible exception of cocaine, all the compounds listed in Table 1 can be classified as phenylalkylamines. Because amphetamine is considered the prototype stimulant, other compounds that have similar chemical structures and similar physiologic effects are often termed ‘amphetamine’ (Figure 2).

Given their structural similarity to endogenous neurotransmitters, it is not surprising that many phenylalkylamines have autonomic nervous system activity, i.e., sympathomimetics, as well as mood-altering effects. Amphetamine and other closely related phenylalkylamines can activate receptors that normally bind catecholamines or serotonin. In addition, amphetamine and related compounds can cause the release of catecholamines and serotonin from nerve endings.22-26 Once released, the endogenous neurotransmitters are free to act on their extracellular receptors. When catecholamine receptors in the brain are activated, myriad effects can result. Neuropsychological effects of catecholamine receptor activation or potentiation by psychostimulants can include: increased alertness, insomnia, euphoria, decreased appetite, and at higher doses, psychosis. Indeed, amphetamine can correctly be called a “psychotomimetic” since high doses can bring about a psychosis very similar to that seen in schizophrenic patients.27,28 In the periphery, activation or potentiation of catecholamine receptors by psychostimulants can result in: vasoconstriction and subsequent hypertension, mydriasis, tachycardia, and other general sympathomimetic effects. The mechanism of action of amphetamine and amphetamine-like psychostimulants involves four major effects:
1. Binding to extracellular catecholamine receptors
2. Inhibition of monoamine neurotransmitter uptake
3. Release of catecholamines from neurons
4. Inhibition of monoamine oxidase

Figure 1. Structure of amphetamine and catecholamine neurotransmitters
Psychostimulants alter neurotransmitter release. Psycho-
stimulants can effectively alter the amount or the rate of
neurotransmitter released by a monoaminergic neuron. The
resulting change in mood or behavior is the summation of
these neuromodulations and is quite complex. At the cellular
level this neuromodulation is due to one or more of the ef-
fects listed above. Amphetamine-like compounds have a wide
range of affinities to catecholamine and serotonin receptors.
The overall pattern of receptor binding for a psychostimulant
is unique for a given stimulant and contributes to the distinc-
tive behavioral effects of a given compound.

In addition to having direct receptor-binding effects, many
psychostimulants inhibit monoamine transporters. Mono-
amine transporter proteins serve to recycle neurotransmitters
after they are released from the neuron and in so doing, ter-
minate the neurotransmitter signal. These same monoamine
transporters are also the targets for antidepressant medica-
tions including the popular drugs fluoxetine (Prozac®),
paroxetine (Paxil®) and sertraline (Zoloft®). Inhibitors of
monoamine transporters block the uptake, or ‘reuptake’, of
neurotransmitters by neurons (Figure 3). This blockade effec-
tively increases the concentration of neurotransmitter in the
synapse, resulting in increased binding of neurotransmitters
to their receptors.

Figure 2. Structures of some common psychostimulants

Structural similarity can be seen among many of the compounds containing the phenylalkylamine structure (compounds shown are from Table 1).
Psychostimulants can also elevate the concentration of neurotransmitter in the synapse by evoking release of neurotransmitters. The release of stored neurotransmitters can be triggered directly when psychostimulants bind receptors present on neurons or can release neurotransmitters indirectly via an exchange mechanism occurring through monoamine transporter proteins. The exchange mechanism or efflux process which can occur via monoamine transporters has been observed with many amphetamine derivatives. This efflux is thought to be a type of ‘reverse-transport’ mediated by monoamine transporters. Finally, amphetamine and amphetamine-like psychostimulants often act as competitive inhibitors of the enzyme monoamine oxidase (MAO). MAO is a mitochondrial enzyme that breaks down monoamine neurotransmitters. Products of catecholamine breakdown include 3-methoxy-4-hydroxymandelic acid (VMA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC). These compounds are commonly-measured metabolites of catecholamines whose formation is due in part or in full to MAO. Inhibition of MAO would thus bring about an expected increase in monoamine neurotransmitters since their breakdown would be impeded.

In summary, psychostimulants can affect neurotransmitter release in at least four ways: by binding extracellular receptors, by blocking monoamine transporters, by evoking neurotransmitter release, and by inhibiting monoamine oxidase.
Phenylalkylamine drugs act at noradrenergic and dopaminergic nerve endings, causing release of norepinephrine in the paraventricular nucleus of the brain. The anorectic effects of amphetamine involve release of norepinephrine from sympathetic nerve endings which causes increases in systolic and diastolic blood pressure, often causing a reflex-decrease in heart rate. At higher doses or overdoses tachycardia or cardiac arrhythmias can occur. Because of these cardiovascular effects, amphetamine is contraindicated in patients with arteriosclerosis, coronary artery disease, hypertension, and closed-angle glaucoma. Interestingly, the commonly used drugs ephedrine and pseudoephedrine are utilized for their vascular effects. By acting directly on alpha-adrenergic receptors in the mucosa of the respiratory tract, pseudoephedrine produces vasoconstriction leading to nasal decongestion.

In the CNS, amphetamine modulates monoamine neurotransmitter release and kinetics. The overall effect of these actions is consistent with known roles of monoamines in the brain. The anorectic effects of amphetamine involve release of norepinephrine in the paraventricular nucleus of the hypothalamus, a brain area central to feeding behavior. Serotonergic systems also play a role in appetite and satiety and these systems are also affected by psychostimulants like amphetamine. In addition to appetite control, amphetamine is also used for its ability to focus attention. The basis for this effect is not well understood. The seemingly paradoxical fact that psychostimulants can act as calming agents in humans seems enigmatic. The effects of amphetamine on attention and alertness are ultimately due to the modulation of normal patterns of central activity. These modulations are brought about by modulating serotoninergic, dopaminergic, and noradrenergic pathways but the precise mechanism of action of amphetamine in ADHD is unknown.

Euphoria, of course, is an important, high-dose effect of amphetamine. The euphoric effects of amphetamine and methamphetamine are very similar to cocaine however unlike cocaine, amphetamine and methamphetamine are taken orally and are metabolized more slowly than cocaine, making it the psychostimulant of choice for many drug users. Centrally, amphetamine and cocaine cause an acute dopamine release in addition to inhibiting neuron dopamine uptake. This release of dopamine in the nucleus accumbens and prefrontal cortex is thought to cause the prominent euphoric and reinforcing effects associated with amphetamine and cocaine. These psychostimulants can produce a significant euphoria, locomotor stimulation, reduced fatigue, sexual stimulation, increased mental attention, and increased sociality. Higher doses can produce tremors and vomiting, as well as tonic-clonic convulsions.

**CONCLUSION**

Psychostimulants are important pharmacological agents used in a variety of conditions including the treatment of obesity, ADHD, narcolepsy, and as decongestants. Psychostimulants typically contain a phenylalkylamine structure that closely resembles that of endogenous monoamines. The actions of psychostimulants are mediated through their ability to bind monoamine receptors and/or modulate monoamine transmitter release. Although psychostimulants have a history of abuse and misuse their therapeutic uses are numerous and significant.

**REFERENCES**

FOCUS: PSYCHOSTIMULANTS

The Use and Abuse of Psychostimulants

SUSAN B GOCK, VICTOR A SKRINSKA

ABBREVIATIONS: DAWN = Drug Abuse Warning Network; ED = emergency department; MDA = methylendioxyamphetamine; MDMA = methylenedioxymethylamphetamine; OTC = over the counter.

INDEX TERMS: drug abuse; psychostimulants.


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Drug use has become a significant medical and social problem in the United States. Toxicological analysis of biological specimens from individuals is generally accepted to be the most objective method for determining drug use and abuse. As a science, forensic toxicology deals with the medico-legal implications of drug use, misuse, and abuse. This may include the following: criminal penalties imposed for the distribution, possession, and use of illicit drugs; assessment of drug impairment in human performance (behavioral) toxicology; assessment of drug toxicity as a contributing factor in the cause and manner of death in postmortem forensic toxicology cases; or detection of drug use in workplace drug testing programs.

Psychostimulants include a diverse class of drugs exhibiting central nervous system stimulant properties, and have a high abuse potential. Drugs in this class include illicit drugs, prescription medications, over the counter (OTC) preparations, and dietary supplements. Clinical indications for therapeutic use include treatment of narcolepsy, attention deficit disorder, and as an appetite suppressant in the treatment of obesity. Pharmacological effects of psychostimulant drugs include the ability to increase alertness, relieve fatigue, decrease appetite, elevate mood, increase confidence, and produce euphoria. Abuse of psychostimulant drugs may lead to tolerance that is exhibited by the need of higher doses of the drug to produce the same desired effects. Consequently, users may try to intensify the drug's positive effects by increasing the drug dosage, taking it more frequently, or changing the route of administration leading to the possibility of drug abuse, misuse, or toxicity. The most common psychostimulant drugs of forensic interest with documented abuse potential are listed in Table 1. These drugs are characterized as either sympathomimetic or hallucinogenic amines with psychostimulant effects. Phentermine, fenfluramine, and diethylproprion are related prescription medications used as appetite suppressants. There is little epidemiological evidence to support the abuse of these three psychostimulant drugs. However, their use is not recommended for individuals with current or past drug abuse problems.

TRENDS IN PSYCHOSTIMULANT ABUSE

Statistics released by the Drug Abuse Warning Network (DAWN) reflect the reporting of specific illicit, prescription, and OTC drugs that are linked to drug abuse in visits to hospital emergency departments (EDs). The data are presented in two issues of The Dawn Report. In 2002, there were an estimated 670,307 ED visits related to drug abuse in the continental U.S. This translates to 0.7% of all visits. Over the past nine years, reports of drug-related ED visits associated with drugs grew at roughly twice the rate of total ED visits. From 1994 to 2002, ED visits related to drug abuse rose 29%, while total ED visits rose only 15%. In 2002, about half (54%) of all visits related to drug abuse involved multiple drugs. Four illicit drugs including cocaine, marijuana, heroin, and methamphetamine accounted for 36% of the drugs involved in these visits.

Cocaine continues to be the most frequently mentioned illicit substance reported to DAWN by hospital EDs nationwide.
Illicit use of psychostimulant drugs reported in 2002 included 199,198 cases for cocaine; 17,696 cases for methamphetamine; and 4,026 cases for methylenedioxyamphetamine (MDMA). From 1995 to 2002, the rate of cocaine-related ED visits increased 33% whereas the rate of methamphetamine-related visits remained stable. Visits involving cocaine exceeded the number of visits for any other illicit drug in 18 of the 21 metropolitan areas monitored by DAWN. Western metropolitan areas including San Francisco, Seattle, San Diego, Los Angeles, and Phoenix had the highest rates of methamphetamine ED visits. According to the National Drug Threat Assessment 2003, prepared by the National Drug Intelligence Center, cocaine is the primary drug threat in the U.S. because of its high demand and availability, expanding distribution to new markets, high rate of associated toxicity issues, and relation to violence.\(^3\)

According to 2002 estimates from DAWN, there were almost 39,000 ED visits related to drug abuse involving amphetamines and methamphetamine. In more than half of these visits the age of the patient was 18 to 34.\(^1,4\) In the report, the category of amphetamines included dextroamphetamine, methcatinone, and methylenedioxyamphetamine (MDA). Visits involving the amphetamines and methamphetamine increased 54% between 1995 and 2002.\(^3\) Data also show that methamphetamine abuse, which was predominately located on the west coast of the U.S. may be spreading eastward. This was demonstrated by an increase in ED visits for amphetamines and methamphetamine in several metropolitan areas in the Midwest, South, and Northeast. However, even though there was an increase, the overall numbers of related visits in these areas remained low. More than 60% of the amphetamine and methamphetamine visits also involved other drugs. Marijuana, alcohol, cocaine, benzodiazepines, opioid pain relievers, and heroin were the most frequently reported substances in combination with amphetamines and methamphetamine.

The term ‘club drugs’ is used to describe illicit recreational drugs that have gained popularity in recent years at nightclubs or large dance parties called ‘raves’.\(^5,6\) Club or rave drugs include stimulants, depressants, and hallucinogenic substances frequently taken for their psychedelic and/or euphoric effects to enhance dancing, auditory, and visual perceptions. Drugs in this class include the following: lysergic acid diethylamide (LSD), gamma-hydroxybutyrate (GHB), ketamine, flunitrazepam (Rohypnol), and MDMA or ecstasy. For the year 2002, club or rave drugs were implicated in approximately 1.2% of all ED visits related to drug abuse that were reported to DAWN.\(^6\) Some of these visits involved multiple club drugs, that were frequently used in combination with alcohol, marijuana, cocaine, and heroin. The incidence of ED visits involving MDMA is increasing based on data provided to DAWN.\(^1,6\) In 1994 MDMA was mentioned 253 times. In 2001 the number of times MDMA was mentioned increased to 5,542 and then subsequently declined to 4,026 in 2002. In 2002, MDMA was the most common club drug detected in drug related ED visits, with 75% of these visits involving patients 26 years or younger.

Methylphenidate (Ritalin®), a schedule II substance, is associated with patterns of abuse similar to other psychostimulants.\(^7,8\) Since it produces many of the same pharmacological effects as cocaine or the amphetamines, a high potential for abuse of this drug also exists. The primary legitimate medicinal use of methylphenidate is for the treatment of attention deficit hyperactivity disorder (ADHD) in children. The increased therapeutic use of this drug for treatment of ADHD is paralleled by an increase in the abuse of the drug among adolescents and young adults due to its increased availability. A recent survey of Wisconsin schools found that most schools did not control how Ritalin was stored or dispensed on school property, making it easy to steal, give away, or sell the drug.\(^7\) Approximately 16% of the students surveyed reported that they had been asked to sell, give, or trade their Ritalin to other students. This pattern of abuse is characterized by increasing

<table>
<thead>
<tr>
<th>Table 1. Common psychostimulant drugs of forensic interest</th>
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</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
</tr>
<tr>
<td>Amphetamine</td>
</tr>
<tr>
<td>Cocaine</td>
</tr>
<tr>
<td>Ephedrine</td>
</tr>
<tr>
<td>Methamphetamine</td>
</tr>
<tr>
<td>Methyleneoxyamphetamine (MDA)</td>
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<tr>
<td>Methyleneoxymethamphetamine (MDMA)</td>
</tr>
<tr>
<td>Methylphenidate</td>
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</tbody>
</table>

FOCUS: PSYCHOSTIMULANTS
doses, frequent episodes of binge use followed by depression, and the desire to continue the use of the drug despite medical and social consequences. The abuser may change the route of administration of the drug from oral to snorting or intravenous injection to intensify the effects.

Recent years have seen an increase in the use of dietary supplements, not only to promote good health, but also as a means of obtaining a ‘natural’ legal high. Stimulants are the most commonly abused dietary supplements used for this purpose. Manufacturers of stimulant dietary supplements market them as natural and safe alternatives for enhanced mental alertness, weight loss, bodybuilding, and athletic performance enhancement. See the table in reference 10 for information on common dietary supplements. Most stimulant dietary supplements contain one or a combination of the following ingredients: ephedrine, pseudoephedrine, synephrine, caffeine, or yohimbine. Herbal Ecstasy®, Metabolife®, and Xenadrine® are examples of OTC dietary supplements containing ephedrine as the selected stimulant. Even though these products are probably safe in most cases when used as directed, a potential for abuse and toxicity still exists as with the other illicit, prescription, and OTC psychostimulants. According to the Toxic Exposure Surveillance System of the American Association of Poison Control Centers, ephedrine products accounted for 77% of the cases of abused or misused dietary supplements in 2002. Due to the increased abuse of stimulant dietary supplements with potential risk of serious adverse events including arrhythmias, seizures, heart attacks, and strokes, the FDA prohibited the sale of all dietary supplements containing ephedrine as of April 12, 2004.

PHARMACOKINETICS OF ILLICIT PSYCHOSTIMULANTS

Cocaine, an alkaloid, is a naturally occurring central nervous system stimulant found in the South American plant Erythroxylon coca. For medicinal use as a local anesthetic, cocaine is administered topically as a hydrochloride in 10% to 20% solutions for the membranes of the throat and nose or in 1% to 4% solutions for ophthalmologic procedures. Street forms of cocaine are sold as either hydrochloride salt or crack. Crack is cocaine that has been processed from cocaine hydrochloride to a free base for smoking. Common routes of administration include intranasal, intravenous, or smoked. Oral is not the preferred route of administration because, when taken orally, the first-pass effects result in low drug bioavailability and reduced euphoric effects due to inefficient delivery to the brain. The intravenous route of administration produces 100% drug bioavailability whereas bioavailability by the intranasal and smoked routes can be quite variable. Due to the convenience of administration, and the rapid, intense onset of effects from the smoked route, intranasal and smoked routes are most commonly used for cocaine self-administration.

Cocaine is metabolized to benzoylecgonine and ecgonine methyl ester, the two major metabolites, by different mechanisms. Cocaine is metabolized in the blood to benzoylecgonine via spontaneous hydrolysis at a physiological and alkaline pH and metabolized to ecgonine methyl ester via enzymatic hydrolysis by pseudocholinesterase and liver esterases with the reaction rate being dependent on drug concentration. Both benzoylecgonine and ecgonine methyl ester are further metabolized to ecgonine. When cocaine is used in combination with ethyl alcohol, a pharmacologically active metabolite, cocaethylene (ethylcocaethylene), is produced by the transesterification of cocaine with ethyl alcohol. Therefore, in cases of simultaneous alcohol and cocaine use, cocaethylene concentrations should also be considered when interpreting results for assessment of toxicity. Anhydroecgonine methyl ester has been identified as a unique metabolite in the post-mortem blood and urine specimens of persons after smoking cocaine. Benzoylecgonine can be detected in blood within 15 to 30 minutes after intravenous, intranasal, and smoking routes of administration. Detection of benzoylecgonine in the urine can provide a means of estimating an approximate window of time of the use of cocaine. Benzoylecgonine can be detected in urine for two to four days after use of cocaine depending on dose, frequency of use, urine pH, and clearance. Detection has been reported seven to sixteen days after chronic compulsive cocaine use.

Ecstasy or MDMA is the most prominent member of the methylenedioxy-substituted amphetamines (hallucinogenic amines) to gain popularity for illicit recreational drug use. At normal doses, the effects include mild to moderate central nervous system stimulating effects as well as enhanced feelings of empathy, closeness, and response to intimate touch, which is why it is also classified as an empathogen-entactogen. Currently, there is no legitimate approved therapeutic use for MDMA in the U.S. The manufacture of MDMA is typically in clandestine laboratories, primarily in Western Europe where it is easier to obtain the precursor chemicals. It is estimated that 80% of the MDMA consumed worldwide is produced in Belgium, Luxembourg, and the Netherlands. The drug is typically supplied in tablet form with an embossed logo, usually white but also available in a variety of colors. Most frequently, MDMA is administered orally in tablet or capsule form in doses of 100 mg to 150

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mg. A popular variation on oral ingestion is ‘parachuting’, in which a tablet is crushed, wrapped in tissue paper, and swallowed for more rapid absorption. This is sometimes supplemented with an uncrushed tablet to achieve both rapid onset as well as a sustained effect.

The onset of action following oral administration is 30 to 60 minutes. MDMA has a half-life of approximately seven hours and undergoes N-demethylation to the active metabolite MDA, which is the metabolite usually detected in blood. Ilicitly produced MDMA is a racemic mixture. The enantiomers exhibit differences in pharmacological activity as well as affinity for the enzyme responsible for their metabolism. MDMA also exhibits nonlinear pharmacokinetics suggesting that beyond a certain threshold, small increases in dose may result in larger increases in blood concentrations with greater risk of toxicity. About 65% of the dose is eliminated in the urine as parent drug and 7% as MDA. Mono- and di-hydroxy metabolites are excreted in the urine as conjugates.

Amphetamine and methamphetamine have limited legitimate pharmacologic use. Methamphetamine is the more potent of these two drugs, producing greater central nervous system effects and having a longer duration of action, most likely due to its greater ability to penetrate the central nervous system (CNS). Current therapeutic uses of these drugs are for the treatment of narcolepsy, obesity, and ADHD. Amphetamine can also be detected as a metabolite of other drugs including fenethylline, fenproporex, and methamphetamine. Illicit preparations of amphetamine usually contain a racemic mixture. Clandestine laboratories use phenylpropanolamine as a precursor to amphetamine manufacture. Because of its ease of manufacture and ready availability, methamphetamine has become the sympathomimetic amine of choice among clandestine producers. In the U.S., methamphetamine is the most frequently encountered clandestinely produced controlled substance. Methamphetamine is easily synthesized from ephedrine. In the U.S., l-methamphetamine (under the label l-deoxyephedrine) is the active constituent of the OTC decongestant Vicks Inhaler containing approximately 50 mg of drug. This isomer is reported to have less CNS activity and greater peripheral sympathomimetic activity than the d-enantiomer. l-Methamphetamine can also be detected as a metabolite of selegiline. d-Methamphetamine is a legal prescription drug (Desoxyn) and a prominent form in many illicit methamphetamine drug preparations available as a water soluble, white, crystalline powder (methamphetamine hydrochloride). Determination of the enantiometric ratio can be useful in determining drug source as illicit, diverted, or licit.

Common routes of administration include oral, intranasal, and intravenous. Smoking remains a minor route of administration compared to the others. Methamphetamine users feel a short yet intense ‘rush’ when the drug is initially administered. In abuse, there is a progression following the start of use, from oral or intranasal routes to intravenous use of the drug. At higher doses, particularly following intravenous use, users typically report intense exhilaration and euphoria, elevated self-esteem, extreme wakefulness, rapid flow of ideas, and increased physical and mental capacity. These effects are perceived as positive and generally encourage repeated administration and produce a binge-type pattern of use. In the binge-type pattern of use, the initial positive effects may be followed by restlessness, irritability, and possibly paranoid psychoses that reinforce the continued use of the drug to maintain the ‘high’ which eventually will lead to tolerance and psychological dependence.

Methamphetamine undergoes phase I metabolism by N-demethylation to amphetamine, its major active metabolite via the cytochrome P450 2D6 isoenzyme system. Amphetamine is metabolized to a variety of metabolites, including norephedrine and p-hydroxyamphetamine, both of which are pharmacologically active and may contribute to the effects of the drug, especially during chronic usage. Accumulated hydroxylated metabolites have been implicated in the development of amphetamine psychosis. Under normal conditions, up to 43% of a dose of methamphetamine is eliminated unchanged in the 24-hour urine, with about 4% to 7% as amphetamine. Elimination of the sympathomimetic amines is highly pH dependent. Urinary acidification to a pH less than 5.6 decreases the plasma half-life from 11 to 12 hours to 7 to 8 hours whereas alkalization increases the half-life to 18 to 34 hours.

PSYCHOSTIMULANT TOXICITY

In toxic doses, the psychostimulants begin to produce unpleasant CNS symptoms including anxiety, agitation, hallucinations, delirium, seizures, and death. High-dose, long-term use of stimulants can induce an acute psychotic state in previously healthy individuals. CNS-induced abnormalities, seizures, or muscular hyperactivity may induce hyperthermia. Secondary rhabdomyolysis may also be seen. Cardiovascular manifestations include hypertension, tachycardia, arrhythmias, and myocardial ischemia. Cerebrovascular accidents are precipitated by elevated blood pressure or drug-induced vasospasms.
The clinical picture of stimulant intoxication also includes a wide array of psychiatric symptoms including schizophrenic symptoms, manic-like states, psychoses, depressions (especially during withdrawal), and various types of anxiety conditions including panic states. Psychotic symptoms usually arise with chronic abuse but may also appear acutely with large doses of stimulants. With high doses of stimulants, symptoms of extreme anger in conjunction with aggressive behavior can also be a catalyst for both violence and murder and is especially seen in cases of methamphetamine and cocaine intoxication.  

Many factors must be considered when interpreting psychostimulant concentrations in blood for an assessment of toxicity since clinical and postmortem studies clearly show that therapeutic, toxic, and lethal concentrations overlap. Tolerance, sensitization, and specimen stability problems are factors complicating the correlation of blood concentrations with assessment of toxicity. Many pathological conditions may predispose an individual to toxicity and possibly death at lower than expected blood concentrations. Sudden death that is attributed to the complications of methamphetamine and cocaine abuse is most commonly associated with cardiovascular effects (disturbances in heart rhythm, heart attacks), but may also be associated with respiratory failure, and neurological effects (strokes, seizure). Seizures are often associated with stimulant abuse but tend to occur only at higher doses. Convulsions are a common sequela to long-term and high dose use that is associated with typical abuse patterns.

Cocaine-induced excited or agitated delirium is an example of a toxic response to cocaine that is frequently associated with death. This syndrome is associated with severe hyperthermia, extreme agitation and delirium, respiratory arrest, and sudden death. These individuals exhibit extreme strength in combination with bizarre and/or violent behavior.

Use of methamphetamine can cause damage to the brain that is detectable months after the use of the drug. The damage to the brain is similar to damage caused by Alzheimer’s disease, stroke, or epilepsy.

Rave party attendees who ingest MDMA are at risk of dehydration, hyperthermia, and heart or kidney failure. These risks are due to a combination of the drug’s stimulant effect that allows the user to dance for long periods of time in the hot and crowded environment of rave parties. The combination of crowded all-night dance parties and MDMA use has been reported to cause fatalities.
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Measurement of 3,4-MDMA and Related Amines in Diagnostic and Forensic Laboratories

VICTOR A SKRINSKA, SUSAN B GOCK

The phenylalkylamine derivatives, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy, XTC, Adam), 3,4-methylenedioxymethamphetamine (MDEA, MDE, Eve), and 3,4-methylenedioxymethamphetamine (MDA), are psychostimulants with hallucinogenic properties. MDA is also a metabolite of both MDMA and MDEA. These drugs are ring-substituted amphetamine derivatives that produce hallucinogenic, entactogenic ('love drug'), and stimulating effects.1-3 MDMA was initially developed as an appetite suppressant, however, its use as a therapeutic drug has been very limited.4 Because of its effects as a hallucinogenic psychostimulant with relatively low toxicity, it has emerged over the last two decades as a common recreational psychostimulant or ‘club drug’ at ‘raves’.5 MDMA, MDEA, and MDA are often referred to as ‘rave’ or ‘designer’ drugs. They are produced in clandestine laboratories and have an increasing presence on the illicit drug market worldwide. Significant adverse health effects have been reported that include: serotonin neurotoxicity, severe psychiatric disorders, renal failure, malignant hyperthermia, hepatitis, rhabdomyolysis, and disseminated intravascular coagulation.6-8 A number of fatal outcomes associated with severe MDMA intoxication have been reported.9-12

Abbreviations: Adam = 3,4-methylenedioxymethamphetamine; ecstasy = 3,4-methylenedioxymethamphetamine; Eve = 3,4-methylenedioxymethamphetamine; GC = capillary gas chromatography; GC/MS = gas chromatography/mass spectrometry; HPLC = high performance liquid chromatography; MDA = 3,4-methylenedioxymethamphetamine; MDE = 3,4-methylenedioxymethamphetamine; MDEA = 3,4-methylenedioxymethamphetamine.

INDEX TERMS: drug testing; hallucinogenic drugs.


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The analysis of MDMA, MDEA, and MDA can be broken down into several categories. The first is the need to identify the presence of the drugs in tablets that are seized and suspected to contain illicit drugs. The second is the need to detect ‘rave’ drugs onsite with the intent to determine recent use of the drugs. The third category is the typical laboratory drug screen used to determine either recent or chronic exposure to the drugs. And finally, the fourth category is forensic analysis of postmortem specimens for the presence of the drugs. The specimens, methodology, and instrumentation vary with each of the categories. Table 1 summarizes the methods that have been developed and reported for these categories.

ANALYSIS OF TABLETS

Tablets containing MDMA and other psychostimulants are prepared in clandestine laboratories worldwide. The tablets
vary in size and typically have logos such as a pitbull, sparrow, butterfly, ‘e’, or ‘X-files’ imprinted on the tablets.\textsuperscript{13} The concentration of the active ingredients varies widely even among tablets from same origin.\textsuperscript{14} The excipients or inert ingredients found in tablets include glucose, sorbitol, and cellulose.\textsuperscript{15} Despite variation in concentration of the active ingredients, analysis of the tablets is helpful in identification of the clandestine laboratory that manufactured them. A number of analytical techniques have been applied to the characterization of the seized tablets. Raman spectroscopy of the active components and the excipients in tablets has been successfully used to identify tablets from the same source based on the state of hydration and the drug/excipient ratio.\textsuperscript{15-18} Another approach is analysis of impurities and byproducts of synthesis by gas chromatography/mass spectrometry (GC/MS), capillary gas chromatography (GC), or high performance liquid chromatography (HPLC).\textsuperscript{19,20} Isotopic analysis of the tablets for the ratios of deuterium, carbon 13, and nitrogen 15 in the active ingredients has been reported as a characteristic that is unique to the site of manufacture and may be a reliable method of fingerprinting the tablets.\textsuperscript{21,22} Capillary zone electrophoresis with ultraviolet detection is a rapid method suitable for routine analysis of MDMA content in tablets.\textsuperscript{23}

ONSITE DETECTION OF PSYCHOSTIMULANTS

When individuals at the scene of a ‘rave’ party, accident, or crime are taken into custody, the need arises for a rapid onsite detection method for MDMA and related drugs. Some immunoassays that have been developed for detection of methamphetamine have high cross reactivity with MDMA and MDA, which make the assays potentially suitable for onsite screens where abuse of psychostimulants is suspected.\textsuperscript{24} Procedures have been reported for onsite analysis of saliva and sweat.\textsuperscript{24-27} The concentrations of MDMA and MDA in saliva have pharmacokinetic parameters that are similar to plasma, thus demonstrating that saliva is a useful and less invasive alternative to analysis of plasma.\textsuperscript{28} Studies have shown that individuals taking a single 100 mg dose of MDMA consistently have detectable levels of MDMA in both sweat and saliva after 1.5 hours. After six hours, most individuals remain positive; however, the number of false negatives begins to increase significantly to almost 20%.\textsuperscript{25} Drugwipe\textsuperscript{\textregistered} has been successfully applied to onsite screens of both saliva wiped from the tongue and sweat collected from armpits.\textsuperscript{26,27} The drug may be quantified and cutoff limits established with a hand photometer, Drugread\textsuperscript{\textregistered}.

DETECTION OF ILLICIT PSYCHOSTIMULANTS IN DRUG SCREENS

Laboratory drug screens for detection of MDMA, MDEA, and MDA typically measure the presence of these substances in plasma, urine, saliva, or hair.\textsuperscript{29-31} The methods used for detection range from relatively rapid basic immunoassays to the

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<th>Specimen</th>
<th>Immunoassay</th>
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<td>Vitreous humor</td>
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more sophisticated and more labor intensive methods such as liquid chromatography with tandem mass spectrometry (LC/MS/MS). Most methods developed for the analysis of drugs in hair use GC/MS instrumentation to obtain the necessary sensitivity. Hair samples range in weight from 10 to 50 mg. Other methods that have been successfully applied to hair analysis for MDMA include capillary electrophoresis, radioimmunoassay, HPLC, and ion mobility spectrometry.

**FORENSIC ANALYSIS OF PSYCHOSTIMULANTS**

Analysis of postmortem specimens for the presence of psychostimulants such as MDMA, MDEA, and MDA typically involves extraction of the drugs from tissues including liver, muscle, and brain as well as from urine, central blood, peripheral blood, and vitreous humor. Varying degrees of putrefaction and postmortem redistribution of drugs further complicate the analysis. While hair and urine are suitable forensic specimens to determine the presence of the drugs, peripheral blood and vitreous humor are reported to provide the best estimate of the blood concentration at the time of death.

There are various analytical techniques available for initial screening, confirmation, and quantification of forensic specimens such as thin-layer chromatography (TLC), HPLC, and GC/MS. TLC is a common initial screening technique when a method capable of detecting a broad-spectrum of drugs in urine specimens is required. Identification is based on Rf value and the color characteristics following exposure to specific staining reagents. The Toxi-Lab A® system for the detection of basic and neutral drugs in urine specimens is able to differentiate sympathomimetic amines such as ephedrine, pseudoephedrine, and phenylpropanolamine from illicit drugs such as amphetamine, methamphetamine, MDMA, and MDA. Sensitivity for most of the drugs in this class using this procedure is approximately 500 ng/mL.

Imunoassays may also be applied to forensic drug screens for the presence of MDMA and related metabolites. However, as mentioned earlier, immunoassay techniques for the detection of amphetamine and methamphetamine have variable amounts of antibody cross reactivity to other drugs. Also, external contact with drugs in powder or liquid form will cause penetration of the hair stock. Nevertheless, it has been shown to be a useful indicator of chronic drug use and is widely accepted as a suitable specimen for drug screens including the detection of MDMA and MDA. All methods for hair analysis require digestion of the hair sample followed by extraction of the drugs. The accepted cutoff for a positive hair sample is 0.1 ng/mg.

**Imunoassays** for amphetamine and methamphetamine generally have high cross reactivity with related drugs and have been successfully applied to urine screens for the detection of MDMA and MDA. The assays have sufficient sensitivity for reliable detection of the drugs at the established cutoff of 500 ng/mL and agree well with results confirmed by GC/MS. The high cross reactivity of immunoassays with structures similar to amphetamine and methamphetamine may result in false positives for MDMA when other substances such as ephedrine or pseudoephedrine are present.

Capillary electrophoresis with electrochemical and fluorescence detection has been successfully applied to the analysis of MDMA in urine with detection limits of 4 ng/mL with an electrochemical detector and 50 ng/mL with a fluorescence detector. Chromatographic techniques such as HPLC with fluorescence detection have been used for measurement of MDMA in plasma and urine with a detection limit of 25 ng/mL. Mass spectrometry methods including GC/MS and LC/MS/MS have been reported for the analysis of plasma and saliva for MDMA and its metabolites with detection limits of 6 ng/mL and 2 ng/mL, respectively.

Hair analysis has been studied as a specimen that may be useful for determination of past chronic exposure to illicit drugs. Hair is a complex structure that grows approximately one cm/month. During its growth, hair is exposed to substances present in the capillary blood circulation near the follicle and substances excreted in sweat at the base of the follicle. Drugs in contact with hair penetrate and embed in the core of the hair stock and remain in the stock for an extended period of time. In the hair stock, drugs are relatively protected from the environment; however, extensive washing of the hair will cause some loss of the embedded drugs.

Analysis of hair provides a historical perspective that suggests chronic abuse of the drugs. After a single 100 mg dose of MDMA, the concentration of the drug peaks at 1.5 hours after intake with concentrations ranging from 135 to 233 ng/mL in plasma and from 1729 to 6510 ng/mL in saliva. After 24 hours, the MDMA levels in plasma and saliva decrease to mean concentrations of 14 ng/mL and 126 ng/mL, respectively. In urine, significant concentrations of MDMA are detectable up to 48 hours yielding a positive screen in urine while the results for plasma and saliva are negative.

Immunoassays for amphetamine and methamphetamine generally have high cross reactivity with related drugs and have been successfully applied to urine screens for the detection of MDMA and MDA. The assays have sufficient sensitivity for reliable detection of the drugs at the established cutoff of 500 ng/mL and agree well with results confirmed by GC/MS. The high cross reactivity of immunoassays with structures similar to amphetamine and methamphetamine may result in false positives for MDMA when other substances such as ephedrine or pseudoephedrine are present.

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structurally related sympathomimetic amines including pseudoephedrine and ephedrine. Antibody cross reactivity is variable and dependent on both the concentration of the structurally related analyte present in the specimen as well as the source of the antibodies used for detection. Higher levels of antibody cross reactivity occur with polyclonal antibody assays in comparison to monoclonal antibody assays. Monoclonal antibody assays are more specific and exhibit less cross-reactivity to structurally related compounds and should be used when high selectivity is desired. Application of immunoassay techniques for the analysis of postmortem specimens poses a problem due to decomposition occurring during the postmortem interval. This may result in the production of biogenic amines such as beta-phenylethylamine or tyramine that have the potential to produce a false positive with amphetamine immunoassays due to the cross reactivity with these analytes. Due to the lack of specificity associated with immunoassays for identification of the specific psychostimulants present in the sample, confirmation of positive immunoassay results should be made using an alternate analytical methodology. GC/MS analysis with selective ion monitoring is the analytical methodology routinely utilized for drug confirmation and quantification. Derivatization of the drugs with heptafluorobutyric anhydride, pentafluoropropionic anhydride, or trifluoroacetic anhydride prior to analysis improves chromatographic behavior and reduces fragmentation so that higher mass fragments can be used for GC/MS selective ion monitoring which allows a more definitive identification and confirmation of the drugs.

CONCLUSION
A wide range of analytical methods have been developed for analysis of MDMA and related psychostimulants. Analysis by GC/MS is the technique that has been reported the most often and has been applied to the widest range of specimen types. No doubt this is due to the high specificity combined with high sensitivity that is found in GC/MS applications. However, there are a number of alternate methods using technologies such as immunoassay, HPLC, and electrophoresis that have sufficient sensitivity and specificity for detection of MDMA in routine screening applications in the laboratory. Furthermore, these alternative methods are more easily automated and more suitable for high volume applications.

REFERENCES


FOCUS: PSYCHOSTIMULANTS

Continuing Education Questions

SPRING 2005

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

NOTE: There may be more answer spaces on the answer sheet than needed. If so, leave them blank. Make sure the number of the answer space being filled matches the number of the questions being answered.

LEARNING OBJECTIVES
After reading the three Focus: Psychostimulants articles in this issue, the reader will be able to:
1. Describe the physiologic actions for amphetamine.
2. Describe the behavioral effects of some common psychostimulants.
3. Identify several therapeutic uses for psychostimulants.
4. Describe the mechanisms of action of amphetamine.
5. List symptoms associated with high doses of amphetamine.
6. List the common psychostimulants of forensic interest.
7. List the illicit psychostimulants that show an increasing trend of abuse in the United States.
8. Describe the composition of crack.
9. List the common routes of administration of cocaine.
10. Identify the major metabolites of cocaine, MDMA, and methamphetamine.
11. Describe symptoms related to toxic doses of cocaine, MDMA, and methamphetamine.
12. List the drugs that are commonly referred to as ‘designer’ or ‘rave’ drugs.
13. Describe the adverse effects of MDMA and related drugs caused by chronic intoxication.
14. Describe specimen collection for on site screening for MDMA.
15. List suitable analytical methods for analysis of MDMA and related amines in tablets, saliva, sweat, plasma, urine, hair, and vitreous humor.

CONTINUING EDUCATION QUESTIONS
1. Which of the following physiologic actions is NOT attributed to amphetamine?
   a. Euphoria
   b. Appetite suppression
   c. Hypotension
   d. Tachycardia

2. Which of the following behavioral effects is NOT associated with common psychostimulants?
   a. Locomotor suppression
   b. Sexual stimulation
   c. Increased sociality
   d. Increased mental attention

3. Amphetamine is used in the treatment of all of the following disorders EXCEPT:
   a. obesity.
   b. narcolepsy.
   c. attention-deficit hyperactivity disorder.
   d. nasal congestion.

4. The mechanisms of action of amphetamine include all of the following EXCEPT:
   a. inhibition of monoamine neurotransmitter uptake.
   b. binding to intracellular catecholamine receptors.
   c. inhibition of monoamine oxidase.
   d. release of catecholamines from neurons.

5. An individual exhibiting symptoms of tremors, vomiting, and tonic-clonic convulsions is possibly experiencing:
   a. withdrawal from amphetamine.
   b. high dose amphetamine.
   c. chronic tolerance to amphetamine.
   d. gastrointestinal complication associated with amphetamine.

6. All of the following drugs are likely to be included in a drug screen protocol in a forensic laboratory EXCEPT:
   a. cocaine.
   b. methamphetamine.
   c. ethylenedioxyamphetamine.
   d. methylenedioxymethamphetamine.

7. The most frequently mentioned illicit drug reported in emergency room visits over the last several years is:
   a. cocaine.
   b. methamphetamine.
   c. methylenedioxymethamphetamine.
   d. amphetamine.
8. Crack is composed of:
   a. cocaine hydrochloride.
   b. methamphetamine free base.
   c. methamphetamine hydrochloride.
   d. cocaine free base.

9. The routes of self administration of cocaine include all of the following EXCEPT:
   a. smoked.
   b. oral.
   c. intranasal.
   d. intravenous.

10. Analysis of a blood sample following cocaine administration will include all of the following metabolites EXCEPT:
    a. benzoylethanolacetate.
    b. ecgonine methyl ester.
    c. benzoylecgonine.
    d. ecgonine.

11. At toxic levels, both cocaine and MDMA cause:
    a. fatigue.
    b. nausea.
    c. hyperthermia.
    d. hypothermia.

12. The group of hallucinogenic psychostimulants that are often found at ‘raves’ includes all of following drugs EXCEPT:
    a. MDMA.
    b. MDEA.
    c. MDA.
    d. MDME.

13. The adverse effects associated with chronic use of ‘rave’ drugs include all of the following EXCEPT:
    a. renal failure.
    b. pancreatitis.
    c. malignant hyperthermia.
    d. rhabdomyolysis.

14. Several techniques have been developed for onsite screening of ‘rave’ drugs. The onsite screens require collection of:
    a. urine.
    b. hair.
    c. blood.
    d. saliva.

15. The analytical technique most commonly used for determination of MDMA levels in hair is:
    a. GC/MS.
    b. HPLC.
    c. LC/MS.
    d. TLC.

16. The chemical structures of most common psychostimulants share the features of:
    a. phenyl ring, alkyl side chain, amine.
    b. phenol ring, unconjugated side chain, amine.
    c. phenyl ring, alkyl side chain, carboxylic acid.
    d. phenol ring, alkyl side chain, ketone.

17. An individual is taken to the emergency room where a blood sample is collected and tested for possible cocaine abuse. The toxicology screen shows the presence of benzoylecgonine and cocaethylene. The conclusion is the:
    a. sample is a false positive.
    b. chronic use of cocaine.
    c. use of cocaine and ethanol.
    d. use of cocaine and amphetamine.

18. In recent years, the percentage of emergency room visits associated with abuse of multiple drugs is approximately:
    a. one third.
    b. two thirds.
    c. three quarters.
    d. one half.

19. The best estimate of a blood concentration of a psychostimulant at the time of death is provided by analysis of a postmortem sample of:
    a. hair.
    b. central blood.
    c. peripheral blood.
    d. urine.

20. An individual is taken into custody at a rave. After 36 hours, a drug screen is ordered. Which specimen would give the best indication of abuse of MDMA at the rave?
    a. Plasma
    b. Saliva
    c. Sweat
    d. Urine
Continuing Education Registration Form

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

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

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

Focus: Psychostimulants carries 3.0 hours of basic level P.A.C.E.* credit. This form can be submitted for credit for up to one year from the date of issue.

Print or type carefully.

(01) NAME ______________________________________________________________________________________________

Last                                                          First                                                       Middle

ASCLS membership number __________________________________   Licensure number ______________________________

(02) ADDRESS __________________________________________________________________________________________

(03) CITY________________________(04) STATE/COUNTRY _____________(05) ZIP/POSTAL CODE_________________

(06) DAYTIME PHONE ( ______ )__________________________(07) E-MAIL:______________________________________

(08) CREDIT CARD # _____________________________  TYPE (CIRCLE)    AE     MC     VIS       EXP . DATE____________

Check all that apply

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

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

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

Participant Information
Please circle the most appropriate answers.

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

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

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

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

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

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

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

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

9. What subjects would you like to see addressed in future Focus articles?
ONLINE
With this issue of Trends and Technology, I decided to break with tradition—the tradition of not reviewing manufacturers’ Web sites. I did not want to create the illusion of favoritism or persuasion by featuring a clinical diagnostics Web site, but after so many years of reviewing other types of sites—governmental agencies, personal lab-related Web sites, some association sites of interest, quality-related sites—I began to wonder: why not? As always, I welcome reader input about sites that may be helpful—or not so helpful—to review for other readers (send your ideas to clstt@aol.com). This time, I visited the site of Ortho-Clinical Diagnostics (www.orthoclinical.com), a subdivision of Johnson & Johnson, and found that when I answered the Web survey, I got a timely response to my query, which led to me look at the site from the standpoint of a laboratory scientist.

I have to say that helpfulness is a hallmark of the Ortho-Clinical site. All of its major analyzers and other product lines are clearly labeled as links to the left on the homepage. The information under “Contact Us” is extensive and inviting, and not just an email address that is often found online. If you want to contact these folks, they make it easy. The homepage also has links to technical support (including holiday photos of these people, putting faces to the voices on the phone) and technical documentation, which brought up 123 “Instructions for Use” documents, down to the last calibrator kit. The most impressive fact of this Web site, for me, is that it is all available with a glance at the homepage, including headline news about new products. Ortho appears to be a company dedicated to customer service and response and its site is indeed user friendly.

NEW PRODUCTS
The VITROS 350 Chemistry System offers easy operation, maintenance, and training. Whether you use it as a primary, STAT, or back-up to the powerful VITROS 5,1 FS, the VITROS 350 Chemistry System can perform the work. The VITROS 350 comes complete with enhanced throughput, a broad accessible menu, and new ergonomic design. “Load-and-go” reagent preparation, MicroSlide™ Technology and up to six months calibration stability mean labor can be redeployed for value-added tasks. Minimal instrument maintenance assists with productivity and reduces costs with the system that delivers the right results the first time. Ortho-Clinical Diagnostics studies indicate that the VITROS 350 Chemistry System with enhanced software improves throughput 10% to 25% and time-to-first-result up to 12% when compared to the VITROS 250 System. Visit www.orthoclinical.com for more information on this and other products.

Ortho-Clinical VITROS 350
Maxell Corporation of America, leader in advanced recordable media products, has announced new ultra-durable and ultra-reliable DVD media designed specifically for the medical market. Maxell’s new medical-grade media incorporates the innovative MAXPRO™ Hardcoat technology to produce enhanced DVD-R media that delivers the highest level of data protection for up to twice the archival shelf life. Maxell Medical DVD-R is HIPAA- and DICOM-compliant, and with its superior scratch, dust and smudge resistance and extended archival life, is ideal for critical medical images, patient records, backup, and fixed content storage. Maxell’s 700 MB, 8X speed Medical DVD-R media will be available in March as a single disc in a jewel case and in 50-pack spindles with printable white surfaces.
TRENDS AND TECHNOLOGY

for either thermal or inkjet printers. Pricing will be affordable compared to other backup media. Contact www.maxell.com.

Healthcare organizations now have continuous access to the Joint Commission on Accreditation of Healthcare Organizations' Periodic Performance Review (PPR) on the Joint Commission’s secure extranet, “JAYCO.” The PPR is an integral component of the Joint Commission’s accreditation process that promotes continuous standards compliance through ongoing, internal monitoring. Before making the PPR continuously available, organizations had received access to the PPR tool 15 months after its last triennial survey and had three months to complete it. Those timeframes for access have now been eliminated. The schedule for completing the PPR remains unchanged until January 2006, when organizations will be expected to update the PPR annually. For 2005, the PPR process requires each accredited organization to conduct a mid-cycle self-assessment against applicable Joint Commission standards, develop a Plan of Action to address identified areas of non-compliance and identify Measures of Success for validating resolution of the identified problem areas. Under the usual PPR process, organizations will be expected to share this information with the Joint Commission at the mid-cycle point. Contact Charlene Hill at 630-792-5175 or email chill@jcaho.org.

Tecan introduced several new laboratory automation products at Lab Automation 2005, the pre-eminent industry meeting, held at the San Jose McEnery Convention Center. Tecan’s interactive trade show booth included the latest unique robotic solutions. Tecan’s participation at Lab Automation 2005 demonstrated robotic solutions that deliver speed, efficiency, and reliability for laboratories focused on clinical diagnostics, genomics, proteomics and drug discovery. Contact Greg Porter, Ph.D., at 919-361-5200.

AxSYM Anti-HCV (hepatitis C virus) is a Microparticle Enzyme Immunoassay (MEIA) for the qualitative detection of anti-HCV IgG to HCV recombinant proteins in human serum or plasma containing potassium EDTA, sodium EDTA, sodium heparin, lithium heparin, sodium citrate, or potassium oxalate. Assay results, in conjunction with other laboratory results and clinical information, may be used to provide presumptive evidence of infection with HCV virus (state of infection or associated disease not determined) in persons with signs or symptoms of hepatitis and in persons at risk for hepatitis C infection. Visit www.abbottdiagnostics.com for the 2005 product releases and listings.

FDA APPROVALS

Bayer Diagnostics has received U.S. Food and Drug Administration (FDA) approval for its automated assay for the hepatitis C virus on the ADVIA Centaur® Immunoassay System. Contact Susan Hager at 781-551-7916.

Bio-Rad Laboratories has received marketing clearance from the FDA for its new Multispot HIV-1/HIV-2 Rapid Test. This highly sensitive test kit this year became available in the U.S. and will significantly aid in the diagnosis of HIV-1/HIV-2 (human immunodeficiency virus, types 1 and 2), the virus that causes AIDS. Contact Susan Berg at 510-741-6063 or email susan_berg@bio-rad.com.

Roche announced that its first microarray-based test, the AmpliChip CYP450 Test, has been cleared by the FDA for diagnostic use in the U.S. This test, which is powered by Affymetrix microarray technology, analyses a patient’s cytochrome P450, 2D6, and 2C19 genotypes from genomic DNA extracted from a blood sample. Test results will allow physicians to consider unique genetic information from patients in selecting medications and doses of medications for a wide variety of common conditions such as cardiac diseases, pain, and cancer. This new test allows physicians access to information that could help to prevent harmful drug interactions and to assure drugs are used optimally. Adverse drug reactions cause

BioPlex 2200

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a huge number of hospitalizations in the U.S. The new test also will, in some cases, enable patients to avoid suboptimal or even harmful treatment choices. For patients it is extremely important to know whether pain killers or anesthetics might work differently or not at all for them. More information is available at www.roche-diagnostics.com.

Dade Behring (NASDAQ: DADE) has received clearance from the FDA for the use of its Advanced D-Dimer assay as an aid in the diagnosis of venous thromboembolism (VTE), [deep vein thrombosis (DVT) or pulmonary embolism (PE)]. The clearance included performance data with a defined cutoff value for the Dade Behring BCS® System and Sysmex® CA-1500 System. The assay is also for use on Dade Behring’s BCT® System, and Sysmex® CA-7000 and CA-560 Systems.

ARRANGEMENTS
VWR International announces the recent acquisition of Alpha-Omega Calibrations, LLC. This acquisition expands VWR’s service portfolio to now include instrument calibration services and repairs for customers nationwide. Alpha-Omega Calibrations’ proven process includes a quality manual, well-documented standard operating procedures, a world-class training program for all their technicians, state-of-the-art facilities and equipment, and decades of gravimetric and metrology experience. Service advantages include fast turnaround times, competitive pricing, full traceability to NIST, and fulfillment of ISO and all other compliance record-keeping requirements. Contact Robin Gervasoni at 610-430-7258 or email robin_gervasoni@vwr.com.

Infotrieve Inc, a provider of content software technology and information services, has announced its acquisition of GenSys Software, Inc, provider of the GenSys/ELN™ electronic laboratory notebook for life sciences, chemistry, and other research-intensive industries. The GenSys/ELN will serve as the anchor for Infotrieve’s life sciences electronic research platform, which has been designed to collectively increase the value of organizational content and improve scientists’ existing workflow by enabling links from electronic laboratory notebooks to discovery tools, literature and scientific data, laboratory product information, and integrated retrieval capabilities for literature and laboratory products. Contact Infotrieve Marketing Manager Ian Palmer by phone at 310-445-3038 or via e-mail at ipalmer@infotrieve.com.

International Technidyne has struck a deal with Medical Automation Systems for the MAS RALS to integrate ITC’s IRMA TRUpoint™ analyzer for point-of-care blood gas monitoring with the widely used RALS®-Plus data management system, marketed by MAS. Contact Check Weber at 847-705-1802.

Abbott and Nihon Kohden Corporation announced that they have entered into an agreement for the commercialization of automated hematology diagnostic instruments for use in hospital laboratories and physician offices. Under terms of the agreement, two 5-part differential hematology instruments, which are designed to offer red and white blood cell analysis, will be manufactured by Nihon Kohden and distributed by Abbott under the CELL-DYN Pearl™ brand name. Abbott obtains exclusive distribution rights for the two instruments in the United States and Canada and non-exclusive distribution rights for the instruments in other countries with the exception of China and Japan. Contact Amy Woodworth at 847-935-4755.

Instructions to Authors
Detailed Instructions to Authors can be found on the ASCLS Website (www.ascls.org) by following the Publications links or going directly to http://www.ascls.org/leadership/cls/index.htm, or obtained by contacting the Clin Lab Sci Editorial Office, PO Box 5399, Coralville, IA 52241. (319) 351-2922. cls@ia.net

Questions may be addressed to Ivan Schwabauer, Managing Editor.
SCIENCE GONE WILD

ASCLS 73rd Annual Meeting, July 26-30, 2005
AACC/ASCLS Clinical Lab Expo, July 26-28, 2005
Orlando, Florida

Scientific Sessions Include:
Connectivity to the Electronic Health Record * Six Sigma and LEAN * Laboratory Design * Body Fluid Cellular Morphology * Molecular Detection of Hematologic Malignancies * Technical Advances in Diagnostic Microbiology * Cardiac Markers * Emergency Preparedness Training for the Lab * Neonatal Screening for Metabolic Diseases * Improved POCT Outcomes * Thrombotic Microangiopathies * Automating Transfusion Services * and More!

Roundtables Include:
POCT Issues * Pre-Analytical Effects with Heparin Monitoring * Patient ID and Risk Management * MRSA * Fun in the Classroom * Meeting Planning * Planning a State Legislative Day * and More!

Opening & Closing Keynotes:
Leadership - Disney Style! Discover the business behind the magic.
It's a Jungle Out There! Real world solutions to acheive goals.

Hilton in the Walt Disney World Resort
Orange County Convention Center

Visit www.ascls.org/conferences/2005AM/ for details and online registration.