YEAR-END HIGHLIGHTS:
Departmental Holiday Party
“And a Good Time Was Had by All”
**Clostridium difficile** is a bacterium found in soils and water that causes diarrhea, usually in patients who have been treated with broad-spectrum antibiotics. It is thought that these antibiotics, when given to a patient to treat or prevent an infection, also kill off the majority of the patient’s normal intestinal bacteria, thereby providing a unique environment in which *C. difficile* flourishes. *C. difficile* infection (CDI) in patients is treated by, paradoxically, an antibiotic. In extreme cases, *C. difficile* diarrhea can progress to a potentially fatal condition known as antibiotic-associated pseudomembranous colitis.

Both *C. difficile* diarrhea and pseudomembranous colitis are very serious problems in a hospital setting, where patients often undergo antibiotic treatment. *C. difficile* can transform into a spore that is resistant to most common disinfectants, and can persist outside the body for months to years. Consequently, *C. difficile* spores are easily spread throughout a hospital and potentially to other patients. In fact, viable *C. difficile* spores have been detected on floors, toilets, bedding, mops, and even in cleaning products in hospitals.

The only disinfectant that effectively kills *C. difficile* spores is 10% bleach—which must be prepared daily. For this reason, strict infection control practices must be enforced for patients with CDI. Even with these precautions, for unknown reasons the number of CDIs has increased substantially in the last decade. Furthermore, a *C. difficile* ‘superbug’ has recently emerged that causes severe or even fatal disease among otherwise healthy people with minimal risk factors.

CDI diagnosis is a challenge for clinicians and laboratories alike. At the UCLA Clinical Microbiology Laboratory, CDI has been diagnosed for many years using a test called an enzyme immunoassay (EIA). This test, which is performed on stool samples, detects the toxins produced by *C. difficile* that are responsible for the disease it causes. Over the last 10 years it has been well-recognized that EIA has low sensitivity, detecting only 33-65% of CDI cases according to some reports.

Doctors attempt to make up for this poor sensitivity by adopting a “three negative tests to rule out CDI” adage which is not supported by national recommendations or backed by data found in scientific literature. Because of this practice, in 2010, 27% of the *C. difficile* tests performed at UCLA were repeat tests from single patients. In one case, 9 stool specimens were sent to the laboratory on a single patient in a one-month period!

Data from 2010 indicate that, for UCLA patients, most (89%) CDI is diagnosed from the first stool specimen sent to the laboratory; the remainder is diagnosed from the second or third stool specimen (Figure 1).

**Fig 1. C. difficile testing in 2010 at UCLA**

No CDI was diagnosed at UCLA in 2010 from a fourth, fifth, sixth, seventh, or even eighth stool sample sent to the laboratory for testing (Figure 1). These numbers highlight how CDI testing is being ineffectively used by the current EIA method. Across the United States, similar problems with CDI diagnosis are leading laboratories to use new nucleic acid amplification techniques, sometimes called “molecular tests”. These tests are far superior to the EIA method and can detect 88-96% of CDI cases. The American Society for Microbiology recently issued guidance documents for clinical laboratories, which state molecular tests should be used for the diagnosis of CDI. Importantly, the guidance documents also states that the use of EIA is no longer an appropriate test for CDI detection.

In an effort to improve patient care at UCLA, the Microbiology laboratory is very excited to launch the Meridian Biosciences illumigene® *C. difficile* test on January 3, 2011, for a trial period of six months. During this time, the current *C. difficile* EIA test will be discontinued. At the end of the trial, the impact of the new test method on CDI diagnosis and treatment practices at UCLA will be assessed.
The *illumigene™* assay is a molecular test that detects the *C. difficile* toxin genes. The *illumigene™* test is simple to perform and fits well in the workflow of the UCLA Microbiology laboratory. Stool specimens can be tested in batches of five, allowing random access and the testing of specimens that arrive at the laboratory “late”. Additionally, because the test is much better at detecting *C. difficile* than the EIA method, replicate testing to rule out CDI is no longer needed.

**What will be different with the new test?**

In parallel with launching the new test, the Microbiology laboratory will be instituting new policies for *C. difficile* testing as outlined in Figure 2. These changes are geared towards streamlining the diagnosis of CDI and aiding patient management. The three major changes are:

1) Rejection of specimens that are not liquid or soft (take the shape of the collection container)

   **Only patients with diarrhea should be tested or treated for *C. difficile***. Since its presence can be detected by the new molecular test even in the absence of active disease, a positive test in a patient who does not have diarrhea can be misleading. Furthermore, while treating an asymptomatic patient who has a positive molecular test with antibiotics to kill off their *C. difficile* may seem like a good idea, this does not, in fact, prevent CDI from occurring down the road, and may even lead to the development of CDI.

   Similarly, the new molecular test should never be used to test for a cure, as half of patients have positive tests for up to six weeks after the completion of therapy and resolution of diarrhea. Therefore, the laboratory will not test a new liquid or soft stool specimen unless 10 days (the recommended treatment course for *C. difficile*) or more have passed since the last positive test.

2) Testing will be restricted to one specimen per 7 days

   Given that the molecular test is highly sensitive at detecting *C. difficile*, multiple stool samples are not needed to detect *C. difficile*. At UCLA, using the EIA assay, 89% of patients are diagnosed with CDI on the first specimen submitted, and with the more sensitive molecular test, we anticipate this to increase.

3) Heightened sensitivity of the new test changes treatment guidelines

   Given the high sensitivity of the new test, therapy for patients with diarrhea and negative molecular test for *C. difficile* should be avoided. Due to lack of faith in the previous EIA method, patients with diarrhea but negative EIA test results were often treated. This practice will be discouraged by Infection Control and the Antimicrobial Stewardship team for those patients with negative *C. difficile* molecular test results.

![Fig. 2a. Old UCLA Policy for *C. difficile* testing](image)

![Fig. 2b. New UCLA Policy for *C. difficile* testing](image)
GREAT TEAMWORK!

Department of Pathology staff, family, and friends recently participated in two charity events: the 26th Annual AIDS Walk Los Angeles, and Heart Walk 2010-2011.

AIDS Walk was held on Sunday, October 17, 2010, with the 10K route extending throughout West Hollywood. Over $2.8 million was raised this year for AIDS Project Los Angeles and 21 other groups. Proceeds benefit programs for people living with HIV/AIDS in Los Angeles county as well as efforts to prevent the further spread of HIV.

The weather was a little cool and drizzly, but that didn’t dampen the spirits of the 30,000 participants! The 17 members of the “UCLA–Anatomic Pathology” team included: Heather Boczko, Ginny Bryan, Po Chu Fung, Robert Grefka, Roya Hariri, David Islas, David Jaquez, Alison Primavera, Richard Pucci, Nikki Salami, Saeedeh Shapourifar-Tehrani, Aparche Yang, and Yong Ying.

Through the generosity of numerous donors, the “UCLA–Anatomic Pathology” team raised $2,010! Out of 34 registered UCLA teams, our team raised more funds than all but one other UCLA team, which helped lead UCLA into 28th place out of 1,735 teams for fund-raising! Richard Pucci served as Team Leader, and David Jaquez earned the distinguished honor of being named a Star Walker for raising over $1,000. Congratulations, David!

The participants of the Heart Walk enjoyed better weather the following Saturday, October 23rd, as they completed the 5K walk around the Rose Bowl in Pasadena. Proceeds support projects that include research into heart disease and stroke, and providing life-saving information to those who need it most. Justine Pomakian and Noelle Linke served as Team Captains for the 26 members of the “UCLA Path Walkers” which also included: Ngan Doan, Dorina Gui, Kam Kuo, Milette Mahinan, Patricia Munoz, Martin Oliva, Gena Rafferty, Jill Squires, and Tina Thomas.

The team raised $733 for the American Heart Association.

Congratulations to all of the team members, and thank you to all of our generous supporters!
Nathan Okawa, Core Laboratory Senior Supervisor, was elected in October to a two-year term on the board of the Greater Los Angeles Chapter, Clinical Laboratory Management Association. CLMA is an association of nearly 3,000 clinical laboratory professionals. The mission of CLMA is to provide leadership to the clinical laboratory industry supporting laboratory professionals at any stage of their career. The Association educates and advocates on behalf of members, and plays a leadership role in enhancing the image and increasing the visibility of the laboratory management profession. The local chapter provides forward thinking educational and networking opportunities in order to assist members in achieving excellence in leadership and health-care management. More information can be found at www.clma.org. Find out more about the local chapter by contacting nokawa@mednet.ucla.edu.

Anatomic Pathology Employees of the Month

L to R:
Michael Cutidioc – Cytology
(October),
Grace Guo – Histology
(November),
Ramzi Bawab – Surgical Pathology
(December)

Anatomic Pathology New Employees Q2 FY11

Syndette Fabello, Senior Supervising Clinical Laboratory Technologist – Flow Cytometry & Bone Marrow Lab

Alvin Ramos, Hospital Laboratory Technician III – Surgical Pathology

Sandra Madha, Histotechnologist II – Histology
MECHANISMS BEHIND TRANSPLANT REJECTION
by Elaine Reed, Ph.D, Vice Chair, Research Services Director, UCLA Immunogenetics Center

UCLA researchers Xiaohai Zhang, Enrique Rozengurt, and Elaine Reed have identified a mechanism linked to chronic rejection of heart, lung and kidney transplants. Published in Science Signaling 2010 Nov 23;3, ra85, their findings suggest new therapeutic approaches for preventing transplant rejection.

Chronic rejection is the major limitation to long term survival of solid organ transplants. Chronically rejected organ transplants develop a progressive and insidious vascular disease known as transplant vasculopathy. Histologically, the blood vessels exhibit smooth muscle cell and endothelial cell proliferation which contribute to thickening of the intima of the blood vessels resulting in occlusion of the lumen. Clinical studies in cardiac, renal and lung transplantation have found strong correlations between the presence of donor specific anti-human leukocyte antigens (HLA) antibodies and development of transplant vasculopathy. Patients that produce antibodies that recognize the donor’s HLA are at higher risk for developing this complication. Currently, there are no therapies toreverse the process of transplant vasculopathy and retransplantation is the only treatment. There is a critical need to develop new therapies for the treatment and prevention of transplant vasculopathy.

The Reed laboratory has researched how antibodies that recognize HLA molecules promote transplant vasculopathy. Their research showed that anti-HLA antibodies can contribute to transplant vasculopathy by triggering signals that elicit endothelial cell activation and proliferation. Furthermore, their research shows for the first time that HLA class I molecules partner with integrin beta4 to transduce signals resulting in endothelial cell proliferation and migration. How HLA molecules signal is still a matter of investigation. In order to explain the ability of HLA class I molecules to transduce signals in endothelial cells, the Reed lab investigated the function of integrins in HLA class I mediated signaling. They discovered a physical and functional association between HLA class I and the integrin subunit beta4. Deletion of the cytoplasmic domain of HLA class I, which is required for the association with integrin beta4, suppressed HLA class I signaling. HLA class I required integrin beta4 expression in order to cause protein phosphorylation. Furthermore, knockdown of integrin beta4 abolished proliferation in response to HLA class I antibodies, demonstrating a dependency of HLA class I on integrin beta4 for induction of cell growth. Importantly, the scientists also uncovered a previously unknown role for HLA class I molecules in integrin beta4 signaling, revealing for the first time that HLA class I expression is required for integrin beta4-mediated functions such as migration and ERK phosphorylation.

HLA class I mediated transduction of signals is most likely of importance in the setting of antibody-mediated rejection of allografts, in which antibody-mediated modifications of endothelial cell function are thought to promote the development of chronic rejection and transplant vasculopathy. Associations between the integrin beta4 subunit and HLA class I might also play an important function in tumor angiogenesis and cancer progression. Given the role of the integrin beta4 subunit in promoting the migration, proliferation, invasion, and metastasis of tumor cells, an increased abundance of HLA may promote angiogenesis by augmenting integrin beta4-dependent signaling. Elucidation of the interaction between integrin beta4 and HLA-class I may lead to the development of novel therapeutic strategies for preventing antibody mediated rejection and transplant vasculopathy.

Congratulations to Dr. Michael Cecka, Professor of Pathology, UCLA Immunogenetics Center, the recipient of the 2010 Terasaki Clinical Research Scientist Award. Dr. Cecka was recognized for his research work studying factors that affect the success of kidney transplants.

Dr. Yiping Jin and Dr. Xiaohai Zhang received the 2010 Scholar Award from the American Society of Immunogenetics and Histocompatibility Testing. Dr. Jin’s research focused on how HLA antibodies to HLA class I antigens elicits activation of endothelial cells and contributes to the process of graft rejection. Dr. Zhang’s research studied the proximal signaling events that occur after antibodies bind to the HLA antigens on the donor endothelial cells and mediate proliferation and migration.

Dr. Xiaohai Zhang, an Assistant Researcher in the UCLA Immunogenetics Center, showed that HLA molecules associate with a protein called integrin beta4 on the endothelial cell to trigger signals that cause endothelial cell growth and survival. His work showed that the binding of antibodies to the HLA molecule caused a physical and functional association between HLA class I and integrin beta4. HLA class I required integrin beta4 expression to cause protein phosphorylation and endothelial cell growth. Importantly, Dr. Zhang also uncovered a previously unknown role for HLA class I molecules in integrin beta4 signaling, revealing for the first time that HLA class I expression is required for integrin beta4-mediated functions such as migration and ERK phosphorylation. These studies demonstrate a mutual dependency of HLA class I on integrin beta4 for induction of endothelial cell migration and growth which may be important in promoting transplant rejection and tumor progression.
The blood center at UCLA was experiencing growing pains as it entered the 1990s. Prior to 1990, platelets were collected only on Saturdays and most of the platelet donors were family members of the patients requiring platelet transfusion as part of their medical care. Given the limited size of this pool of donors, and the expanding medical need for platelets, recruitment began to focus on attracting community donors. These efforts were successful and by 1993 the number of eligible platelet donors and units collected had increased considerably. In response to the need for more space, collection activities moved to the 6th floor of 200 Medical Plaza in April of 1994. During the same period, the center was officially renamed The UCLA Blood & Platelet Center to better reflect the expanded scope of operations.

In the new quarters, the collection of blood and platelets, along with donor interviews were finally centralized in a single location. As the 1990s progressed, a major focus of the UCLA Blood & Platelet Center involved safety and compliance to the increasing number of regulations for collecting blood products. The implementation of numerous screening tests increased the safety of the general blood supply and consequently the public’s perception of the safety of blood collection and transfusion improved. Although more units were being collected, patient needs continued to rise at a rate that outpaced donations. It soon became apparent that the Blood & Platelet Center’s efforts would have to expand further to meet the medical demands of UCLA’s patients. Thanks to the vision of Barbara Willahan, the donor center’s manager at the time, blood collection for our patients moved beyond the walls of the hospital with the implementation of the Mobile Blood Drive program in 1999.

In the first year, 1,638 units of whole blood were collected at mobile blood drives, representing about 10% of the total amount collected by the Blood & Platelet Center. By the end of the next decade mobile blood drives accounted for well over 70% of total units of whole blood collected by the Blood & Platelet Center’s overall operations, with more than 17,500 units collected. This increased volume of collections required a threefold increase in the number of the Center’s personnel to meet the workload. In 2005 the acquisition of a Self-Contained Unit Blood Mobile (with three beds) and a burgeoning association with the Los Angeles Unified School District permitted even greater opportunities for the staff to collect needed blood units.

The move of the donor center from 200 Medical Plaza to 1045 Gayley Avenue in Westwood Village had the unfortunate consequence of reduced donations since the new location was no longer in the hospital and a greater distance from the UCLA campus. However, the opening of an additional satellite donor center, conveniently located in the Ackerman Student Union on the A-Level, in April of 2009 reclaimed a great number of former donors and attracted a large and growing population of new donors. The UCLA Blood & Platelet Center was the first donor center in the country to open a dedicated center within the student union on a college campus. An impressive accomplishment of the Blood & Platelet Center is the safeguarding and maintenance of the blood supply by collecting an average of 81% of the hospital’s blood product needs and, in some months, collecting 100% of what is required.

As the UCLA Blood & Platelet Center moves into the second decade of the 21st century, plans are already in place to meet the ever-increasing blood needs of the hospital. Over the next two years, a state-of-the-art electronic donor scheduling system will be implemented. This new tool will increase efficiency for the team at the Blood & Platelet Center, allowing them to focus on collecting the precise blood products needed by patients and permitting donors to schedule their own appointments electronically.

The future promises a robust and proactive approach to collecting blood and platelets and that the UCLA Blood & Platelet Center will continue in its mission to ensure the safety and maintenance of the hospital’s blood supply while accommodating donors and serving the needs of its patients.