



InFocus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology



From humble beginnings...
Mus spretus, also known as the Algerian mouse, is one of the contributors to the C57BL genome.

Image credit: <http://www.sci-news.com/genetics/article00214.html>



The C57BL/6J is the most widely used inbred strain and the first to have its genome sequenced.

Image credit: http://www.nature.com/nature/journal/v420/n6915/fig_tab/420515a_F1.html



The delicate balance of anesthesia; its indisputable need vs its inevitable effects.

Inside:

- ✓ Two new Managers join RARC
- ✓ Laboratory of Comparative Pathology Update

Substrain Differences among C57BL/6 Mice: A Resource for Investigators

The C57BL strain, developed by C.C. Little, originated in 1921 from a single female (C57) and male pair at Harvard University's Bussey Institute¹. Accounting for more than 14% of inbred mouse use, C57BL is the most widely used inbred mouse strain. Its genome contains contributions of Asian *Mus musculus* as well as *Mus spretus*¹. Breeding was transferred to the Jackson Laboratory in 1948 and the mouse was distributed to the National Institutes of Health (NIH) in 1951 and Charles River in 1974². C57BL/6 (B6) is believed to have diverged from C57BL/10 circa 1937 and by the 1970s, multiple C57BL/6 substrains were available, including the C57BL/6J (B6J, Jackson Laboratory) and the C57BL/6N (B6N, NIH). Widespread use of B6J warranted sequencing of the genome began in 1999 and was completed in December 2002 by the Mouse Genome Sequencing Consortium³. The B6N substrain has also become important. It took five years for the International Knockout Mouse Consortium to knock out almost 20,000 protein-encoding genes in

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The Impact of Anesthetics on Brain Development and Cognitive Function in Animal Models

While the use of anesthesia during various procedures on research animals is necessary for both scientific and humane reasons, recent studies have shown that its effects may not be as innocuous and reversible as previously thought. The impact of anesthetics depends on multiple variables, including the species, the age of the subject or stage of brain development, the anesthetic drug used, and the degree of anesthetic exposure¹. Close attention must be paid to the differential effects of anesthetics as these effects have been shown to run the gamut from neurotoxic, to neuroprotective, to neuroenhancing.

Anesthetics act through two principal mechanisms. Many anesthetics, including but not limited to isoflurane, sevoflurane, propofol, benzodiazepines, and barbiturates, act by increasing fast synaptic inhibition in the brain via GABAA receptors^{2,3,4}. Other anesthetics, including ketamine and nitrous oxide, work by inhibiting glutamate activation (excitation) of NMDA receptors^{3,5}. Both sets of drugs have been shown to cause neuronal apoptosis in the brains of immature rats³.

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Preemptive Analgesia Part I: Physiological Pathways of Pain

Alleviating pain and distress is of primary importance in biomedical research. Not only are we ethically and morally compelled to minimize pain and suffering, the production of valid scientific data may also be compromised by inadequate pain alleviation.

In the skin and other superficial structures, pain nociceptors exist as free nerve endings. These nociceptors may be stimulated by mechanical damage, extreme heat or cold, as well as irritating chemical substances or a combination of these stimuli. At the site of injury, the pain receptors convert the stimuli into a neural impulse, a process known as transduction. When tissue damage occurs, the tissue will secrete various chemical substances (such as prostaglandins) and enzymes that increase the transduction of the painful stimuli. This is done by reducing the intensity of the stimulus needed to activate the receptors,

increasing their responsiveness to other stimuli, and increasing transmission of pain impulses along the peripheral nerves. Additionally, it stimulates the secretion of other various substances which increase inflammation, accelerate sensitization, and induce synergy between these pro-inflammatory molecules. Combined, all these changes lead to a profound increase in nociceptor sensitivity leading to increased pain sensation and may lead to the development of chronic pain states.

The impulses created by the peripheral nerves will then travel along nerve fibers to the spinal cord. These first-order neurons enter the spinal cord and synapse with second-order neurons. At the site of synapse, various neurotransmitters are released by the first-order neurons that increase the excitability of the second-order neurons. This also stimulates the release of excitatory amino acids that

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Tracy Livingston Rejoins RARC as First BRBV Facility Manager

We are very pleased to have Tracy Livingston return to RARC as Facility Manager for Weill Cornell's Belfer Research Building Vivarium (BRBV) which is scheduled to open in early 2014. Tracy is a native New Yorker and has been involved in laboratory animal science and husbandry for over 20 years. Tracy began her career as a Veterinary Technician after receiving her Associates in Animal Health Technology degree from LaGuardia Community College here in NYC. Tracy joined MSKCC in 1994 and worked as a veterinary technician until 2001. During this period Tracy obtained her AALAS Laboratory Animal Technician (LAT) and subsequently her Laboratory Animal Technologist (LATg) certification. Tracy is currently working on obtaining her Certified Manager of Animal Resources (CMAR) certification.

Tracy served during the past 8 years as a Facility Manager at the Washington University School of Medicine in St. Louis, Missouri where she was responsible for up to 5 research facilities housing a variety of species and a staff of up to 44 animal care technicians. Tracy served on the Operations Committee formed by the Department of Pathology, which developed the standard operating procedures for maintaining several suites of severely immunocompromised mice free of murine norovirus. She has also held positions at Columbia University's College of Physicians and Surgeons.

Tracy strongly believes in applying a hands-on approach to facility management and mentoring employees. Tracy's commitment to excellence, positive customer service and attention to detail has been her trademark. She approaches each new project with vision, creativity and resourcefulness and has a reputation of being a professional, responsive leader who diligently works hard to foster positive relationships with researchers and the many staff involved in the research process. Tracy takes the time to nurture and mentor her staff, passing on to them the vision to succeed and the pursuit of excellence. She is a manager who truly realizes the importance of team work and positive, proactive change.

Lynn Fleck, Joins RARC as Manager, Cage Wash Operations

Lynn Fleck recently joined RARC as Manager, Cage Wash Operations. In this role she is responsible for the operation of 5 cage wash centers operated by approximately 50 personnel. Lynn replaced Cindy Haab who has relocated to Qatar to help establish Weill Cornell's animal research program in Doha. Lynn is a life-long resident of New Jersey. Growing up on a horse farm she gained a strong work ethic and a love of animals.

Lynn has over 27 years of experience in Lab Animal Science beginning her career in cage wash working in a variety of roles including Animal Care Technician, Veterinary Technician, and Supervisor in a GLP regulated environment. In her last role with Sanofi-Aventis she was responsible for vivarium logistics, equipment and environmental oversight.

Lynn holds a BS in Laboratory Animal Science and is RLATG and CMAR certified. She remains actively involved in AALAS. With a mechanical aptitude and extensive project management experience in facility operations, she also recently took and passed the PMP (Project Management Professional) certification exam administered by the Project Management Institute.

In her free time Lynn loves outdoor activities including hiking and four-wheeling, and drawing in charcoal and graphite. Lynn shared:

"I believe in building people and promoting their best qualities to achieve their goals, with my accomplishments as proof that if you want it you can do it."

Preemptive Analgesia, from pg. 1

prolong the enhanced transmission of signals between the neurons. This enhanced transmission leads to persistent changes in the excitability and sensitization of the cell which has been termed "wind-up".

These second order neurons conduct nerve impulses which ultimately lead to the somatosensory cortex and the reticular formation. The somatosensory cortex is responsible for the discrimination of pain, whereas the reticular formation is involved in the emotive aspect of pain sensation.

Finally the pain impulses descend to the lower neurons which modify afferent nociceptive information. In peripheral tissues, the nervous system responsiveness can be modified via two processes. Peripheral sensitization involves a reduction in the threshold level of cellular firing of the

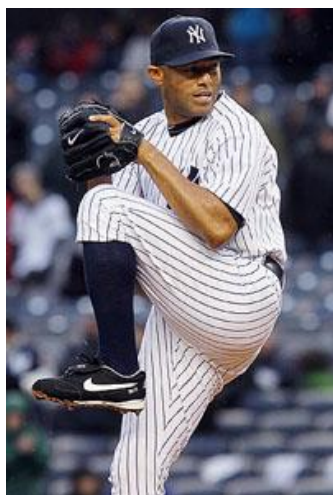
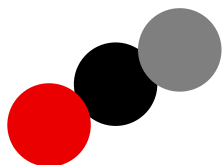
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Tracy Livingston returns to New York as Facility Manager of the new Belfer Research Building Vivarium



CCMP welcomes Lynn Fleck, as new Manager, Cage Wash Operations



Pre-emptive analgesia- addressing a different kind of wind-up.

Image credit: http://espn.go.com/blog/sweetspot/tag/_name/brian-wilson



Substrain Differences among C57BL/6 Mice, *Cont. from pg. 1*



Substrains arise for various reasons—intended and unintended. The following link provides more information and a detailed bibliography on the subject:

<http://jaxmice.jax.org/genetichealth/substrains.html>

B6N mouse embryonic stem (ES) cells⁴. While 129 strain mice were historically utilized to produce ES cells used in mouse knockout technology, strain differences on C57BL/6 backcrosses resulted in trouble identifying the genetic basis for many phenotypes and confounded research results⁵. ES cells were derived using both B6J and B6N mice, but B6N mice had better growth and morphology and were better adapted to ES cell culture conditions⁶. While individual studies have identified key differences between various C57BL/6 substrains [Muller et al⁷, and references therein], several genome-wide analyses have helped to better characterize genotypic and phenotypic differences between the substrains⁸⁻¹⁰.

In the age of functional genomics, early studies in genetic variation between B6J and B6N have identified genetic differences at minimally 10 loci^{6,8,9}, but a new study focusing on mice from four different research centers identified variations in 34 single nucleotide polymorphism (SNPs) in coding regions and two insertion-deletion variants between the strains¹⁰. Fourteen of the coding SNPs are suspected to correspond to observable phenotypes. All B6J substrains studied in 2009 had a deletion of the *Nnt* gene (encoding for nicotinamide nucleotide transhydrogenase) which plays a role in impaired glucose tolerance⁸. The work by Simon et al. confirmed that phenotype and identified 27 additional phenotypic differences between B6J and B6N mice¹⁰. The authors discuss the genes that may contribute to the phenotypic variability between the strains and their findings are summarized below.

In terms of the eye, B6N mice have reduced vision compared to B6J mice¹⁰. In addition, B6N mice have white flecks in the fundus, which are proposed to be due to a carbonyl reductase 1 variant (*Cbr1rd8*). The flecks are not present in B6J mice; B6J mice have more fundic vessels than B6N mice.

There are also differences in the cardiovascular system¹⁰. B6J mice displayed higher systolic pressures, but B6N mice had higher pulse rates. In addition, male B6N mice had lower heart rates under anesthesia and B6N mice overall had a lower normalized heart weight. There was also variability in gas exchange and energy production in the two strains with B6N mice having an increased fat mass¹⁰. Hematological and clinical chemistry parameters showed variations in free fatty acids, iron, alkaline phosphatase, white blood cell

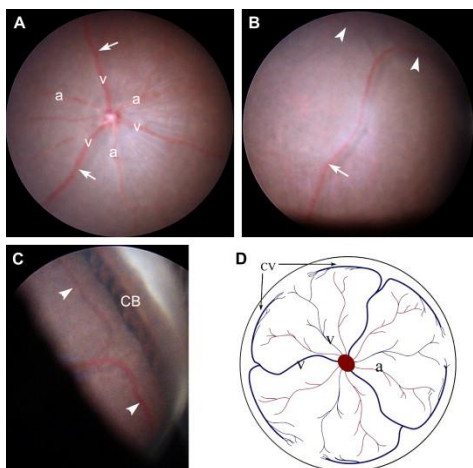
counts, red blood cell counts and hemoglobin values between the two substrains.

Behavioral studies revealed that B6J male mice had higher activity in open field tests, indicative of reduced anxiety compared to B6N mice, but this finding was observed only in mice in two out of the four centers that participated in the study¹⁰. Male B6J mice had higher raised locomotor activity and higher grip-strength. Differences were also noted on 'learning' and 'latency to fall' on a rotarod. In addition, B6N male mice had reduced performance in the Morris Water Maze test for spatial memory. In general, B6J mice had an increase in contact hypersensitivity induced by dinitrofluorobenzene (DNFB), while female mice of both strains had an increased hypersensitivity compared to males. Immune system differences were not well-characterized in this study and there were insignificant differences in the host response to the single pathogen tested (*Listeria monocytogenes*)¹⁰.

Other studies have highlighted the phenotypic differences amongst B6 substrains. Researchers have identified differences in response to cardiac pressure overload, susceptibility to pilocarpine in a seizure model, conditioned fear responses, susceptibility to chronic pancreatitis and other features^{7,11-13}. Of note, even within a single substrain, there may be phenotypic differences based on the environment. One study identified variability in mice in regards to airway responsiveness in an asthma model of B6N mice from different vendors¹⁴. Environmental differences have been shown to produce different experimental results with identical mouse strains¹⁵ and may explain the changes that Simon and colleagues observed in mice from some of the centers, but not others.

Phenotypic differences between B6J and B6N strains are attributed to mutations that have arisen within the lineages and can have consequences to research investigations, but they are valuable to geneticists. It is important for researchers to consider substrain differences when selecting a strain in a new or ongoing study. In addition it is important to have type-matched substrains for control groups, because if they are not sufficiently similar it could introduce a confounding variable into the study¹⁶. While Simon et al studied many anatomical and physiological features, immune system variability, if any, amongst substrains remains to be better characterized. The Simon et al report is an excellent resource for researchers who need to familiarize themselves with B6 substrain differences and select a

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Normal mouse fundus images. Fundus images were taken from a normal 8-week-old mouse using the endoscopic fundus imaging system. (A) Posterior pole of the fundus; (B) Equatorial region of the fundus; (C) Peripheral region of the fundus. ... (D) An overview of mouse retinal vasculature. a, artery; v, vein; CB, ciliary body; CV, collecting vein
Image and caption credit: <http://www.science.direct.com/science/article/pii/S0014483508001929>

**Seeing is not believing
They may look alike but studies
reveal substrain differences in
physiology, anatomy, behavior
and cognition.**



**Register for the November 7th
Webinar:**

**Protect Your Research:
Know Your B6 Mouse**

<http://jaxmice.jax.org/webinar/2013/B6N-JWebinarNov.html>

**You don't have
to manage your own mouse
colony - there is an "App" for
that!**

In 2004, the Mouse Genetics Core (MGC) established the Colony Management Group (CMG) to provide professional assistance in the management of investigators' breeding colonies at the off-site LIC Vivarium. The principal aim of the CMG was to reduce the impact of colony relocation on laboratories required to use this off-campus site. Under the direction of the CMG Supervisor, Dr. Peter Romanienko, the CMG's husbandry staff sets up and maintains breeding cages based on the needs of the user. The staff monitors colony breeding performance by checking births and weaning mice at puberty. If requested, the staff identifies individual animals and harvest tissue for genotyping. Tissue samples can be sent to the user for genotyping in their own laboratory or delivered directly to the MGC's Molecular Biology Group for PCR or Southern Blot analysis. If requested, images of Southern blots and PCR data are sent to investigators electronically. Breeding colony activity is communicated to investigators weekly. Using this weekly report, users instruct the husbandry staff to alter the output of each production colony to fit their research needs.

This Colony Management Service has proven to be immensely popular with investigators at MSKCC. In the first year of operation, 22 laboratories utilized this service. Currently, the CMG's husbandry staff maintains and breeds more than 300 different mouse strains for 42 laboratories. The molecular biology staff performs over 10,000 PCR assays each month. The efficient management of a research animal colony by dedicated CMG staff allows investigators to maintain the minimum number of mice needed for experimental usage. Furthermore, the use of this service allows technicians, students, and fellows in the laboratory to focus on their research rather than spending time caring for animals and aids PI's who use mice in their research program to reduce personnel and animal maintenance costs.

The recent opening of the Phase 2 ZRC Vivarium allowed a portion of the animals housed in the LIC Vivarium to return to main campus. As a result, space is now available at LIC for WCMC investigators who wish to take advantage of the CMG's services. Weill Cornell users interested in using this service should contact the MGC at cmg@mskcc.org to schedule a meeting to discuss their animal service needs.

For a detailed description of the CMG services please go to the following:

WCMC intranet @

http://intranet.med.cornell.edu/research/arclani_ser/vet_services.html?name1=Veterinary+Services&type1=2Active

MSKCC intranet @

http://mskweb6.mskcc.org/tmcf/html_pages/breeding_colony.html

Substrain Differences among C57BL/6 Mice, cont. from pg. 3

particular substrain for their use. Investigators should also consider discussing substrain options with a veterinarian when selecting research subjects.

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Preemptive Analgesia, cont. from pg. 2

free nerve fibers. Central sensitization involves an increase in the excitability of spinal neurons. These two processes lead to an increase in response to noxious stimuli and a decrease in pain threshold. This can ultimately lead to allodynia which is the perception of pain from non-noxious stimuli.

Preemptive analgesia is used to prevent establishment of altered processing of afferent input, which amplifies postoperative pain. This technique is extensively utilized in human and veterinary medicine and has been shown to effectively decrease the level of post-surgical pain. Therefore, immediate postoperative pain is reduced and chronic pain development may be prevented. Additionally, it may decrease anesthetic needs peri-operatively and therefore decrease the potential side effects of these agents.

There are three criteria for the analgesia to be considered preemptive. It must be a treatment that starts PRIOR to surgery and prevents the initiation of "wind-up" and therefore, prevents the establishment of central sensitization caused by the surgical incision during the surgery. Additionally, it prevents establishment of central sensitization caused by the incision and resulting

inflammatory injury during the surgery and initial postoperative period.

RARC strongly recommends the use of preemptive analgesia for all procedures that may cause pain. The type of analgesic given is determined by how painful the procedure is expected to be. Animals subjected to procedures that are expected to produce minimal to mild pain will typically benefit from a pre-emptive local anesthetic and/or pre-emptive non-steroidal anti-inflammatory (eg, meloxicam) or opioid (eg, buprenorphine). Animals subjected to procedures that are expected to produce mild to moderate to severe pain will typically benefit from a preemptive local anesthetic, NSAID, and opioid. Animals will then receive analgesics (NSAID and/or opioid) for 24-72 hours post-operatively, depending on the severity of the pain. Given the highly subjective nature of pain evaluation in rodents, PRN or as needed dosing regimens are not recommended. Please contact RARC's Veterinary Services if you would like assistance in selecting analgesic regimens for planned surgical and painful procedures.

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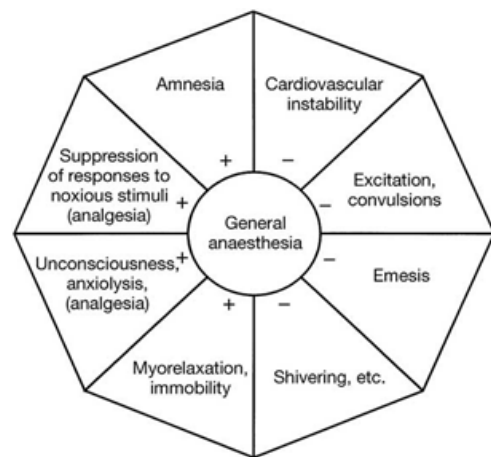
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The Impact of Anesthetics on Brain Development and Cognitive Function, Cont. from pg 1.



The use of anesthesia in neonatal animals has been shown to cause neurodegeneration which may be of concern in neurological or behavioral studies.



Spectrum of clinical effects in general anaesthesia.

Image and caption credit:

<http://www.sciencedirect.com/science/article/pii/S0378427498001593>



Older animals may also exhibit memory impairment attributable to anesthetic use. Studies have shown a decrease in cognitive performance of adult rodents after exposure to isoflurane.

Image credit: <http://www.sciencemuseum.org.uk/antenna/ratbrains/131.asp>

The infant brain is most susceptible to the neurodegenerative effects of anesthetics during the period of synaptogenesis. This developmental period occurs in mice and rats in the early postnatal period (~ 4 to 10 days of age) and in humans between late gestation and ~6 months of age^{3,6}. However, susceptibility of different areas of the brain varies by developmental period¹. Infant (P7) rats exposed to a triple cocktail of midazolam, isoflurane, and nitrous oxide had severe neurodegeneration in multiple brain regions (thalamus and parietal cortex) and displayed long-term spatial learning and memory deficits³. While NMDA receptor antagonists and GABAA receptor agonists alone induce neuroapoptosis in the infant rodent's brain, there appears to be a more profound response if both receptors are affected at once³. In addition to mice and rats, isoflurane has also been associated with neuroapoptosis in neonatal guinea pigs and piglets⁷.

Many anesthetic drugs, including the benzodiazepines, barbiturates, propofol, and ketamine have been shown to cause dose-dependent neurodegeneration⁷. However of the aforementioned drugs, only propofol and ketamine (at higher doses) caused longer term cognitive deficits⁷. Sevoflurane, isoflurane, and propofol have also been shown to cause neurotoxicity via increased formation of reactive oxygen species¹. Even though anesthesia has been linked to a decrease in neurotrophic factor, the exact mechanism of anesthesia-induced apoptosis remains unknown⁷.

Adult and geriatric animals are also affected by anesthesia. Studies have shown a decrease in cognitive performance of adult rodents after exposure to isoflurane^{1,2}. A study in aged rats (18 months old) displayed a long lasting, but reversible, impairment in spatial memory performance after anesthesia with 1.2% isoflurane in 70% nitrous oxide/30% oxygen for 2 hours⁸. However, young rats (6 months old) in the same study demonstrated memory enhancement after anesthesia⁸. These effects lasted up to two months⁸.

Even though anesthesia can be deleterious, some studies have reported beneficial effects of anesthesia. In particular, ketamine, propofol, etomidate and barbiturates have been shown to have neuroprotective effects in adult animals during brain ischemia⁷. The neuroprotective effects of these drugs

have not been studied in developing or immature animals⁷. However, isoflurane and sevoflurane are neuroprotective during periods of ischemia in the immature brain⁷. In addition, nitrous oxide has been shown to protect against excitotoxic damage⁷.

Surprisingly, sevoflurane has been shown to improve cognitive performance in 4-5 month old C57BL6/J mice². While hippocampus-dependent cognitive performance improved from two until eight days post-anesthesia, there was no effect on hippocampal long-term potentiation (LTP)². The mechanism of improved cognition was thought to be related to increased expression of NMDA receptors after anesthesia². These mice were also found to be less anxious after day 6. However, this was interpreted to be related to improved cognition². Alternatively, after isoflurane anesthesia for tail biopsies, mice have been shown to exhibit increased anxiety and decreased activity⁹.

It is evident after this brief review that anesthesia is certainly a variable to consider when performing research in animal models. Even though the literature contains some conflicting information, it is clear that the use of anesthesia in neonatal animals causes neurodegeneration which may be of particular concern in neurological or behavioral studies. In conclusion, even though anesthesia means without sensation or feelings, you should certainly have feelings about the effects of anesthesia on your research.

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Laboratory of Comparative Pathology (LCP) Update: New instruments allow for the longitudinal analyses of comprehensive blood analytes from individual rodents

In the past 12 months the LCP has acquired high-throughput, low-volume hematology and clinical chemistry analyzers and now has a board-certified Veterinary Clinical Pathologist on staff to offer improved clinical pathology testing and interpretation of both diagnostic and research results. These tests are often combined with our anatomic pathology services (necropsy, histopathology, and phenotyping) to offer comprehensive research animal pathology services.

A new hematology analyzer allows rapid measurement of complete blood counts (CBCs) from laboratory animal species. The CBC is useful for characterization of red cell abnormalities, such as anemia, including differentiation into regenerative versus non-regenerative anemia; platelet number and morphology; and, white blood cell (WBC) changes ranging from changes in absolute numbers, such as can occur with leukemia, neoplasia, inflammatory demand, and radiation induced leukopenia, to abnormalities in the ratios or morphology of individual WBCs. Of significant importance, this analyzer is capable of performing a full CBC using as little as 40 μ L of unclotted whole blood. The low volume required makes this analyzer ideal for small laboratory animal species, such as mice and rats, and allows for longitudinal analysis from individual animals. In addition, unlike most human hematology analyzers, the instrument is programmed with species specific analysis parameters, taking into account the different morphologic characteristics of blood cells between species and providing *optimal categorization of red cells, white cells and platelets of commonly used laboratory animal species. Twenty-six parameters are measured and reported by the laboratory when requesting a CBC. We also perform manual examination of the peripheral blood smear to report on the morphology of red blood cells, white blood cells, and platelets, and have a Clinical Pathologist available for consultation, thereby providing comprehensive hematology services.*

The LCP also recently acquired a clinical chemistry analyzer. This instrument performs a variety of routine serum and urine chemistry measurements important to researchers, veterinary staff, and those needing phenotypic characterization of their animals. Currently available tests include enzymes (ALT, AST, ALP, CPK, LDH, GGT, Amylase, Lipase) and concentration measurements of proteins

(total protein and albumin), electrolytes (Na, K, Cl, HCO₃), metabolites (BUN, Creatinine, Glucose, Bilirubin, Cholesterol, Triglycerides, NH₄, T₄, TU) and other elements (Ca, Fe, P, Mg). This instrument can reliably obtain as many as 22 parameters on < 130 μ L of serum. Combining this with the volume requirement of our hematology analyzer makes it possible to offer survival samplings in mice and other small rodents.

If you are interested in our hematology and clinical chemistry services, please do not hesitate to contact our Clinical Pathologist, Dr. Michelle Lephherd, BVSc PhD DACVP (lepherdm@mskcc.org), or our Laboratory Manager, John Sibley (sibleyj1@mskcc.org) or phone them at 646-888-2422.

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The low volume required, as little as 40 μ L of whole blood, makes the LCP's new analyzers ideal for rodents. Longitudinal analysis from individual animals is now possible.



AU680 Chemistry Analyzer

The LCP's new clinical chemistry analyzer performs a variety of routine measurements important to researchers, veterinary staff, and those needing phenotypic characterization.

IACUC Establishes Euthanasia Rack Policy at WCMC to Ensure Compliance

Continued noncompliant use of RARC's automated euthanasia racks has led the IACUC to establish a new policy regarding identification of cages placed on the racks. The policy requires that all cages left on a "Euthanasia Rack" be labeled with an identification card. One of two labeling options can be used.

For newly created cages being sacrificed, a **NOTES card** (found in all animal housing rooms) is placed on the cage with a pre-printed bar code sticker attached and the contact name written on the card. Alternatively, the **primary cage card** is left on the cage.

Signage identifying the correct process for utilizing the euthanasia racks is clearly posted on each rack. Please adhere to these instructions and remind lab staff of this important policy.

