

Research Review

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1st Resident Research Retreat a Big Success

106 enthusiastic participants attended a research retreat hosted by the BAMC Department of Clinical Investigation on 22 Oct 02. The retreat was specifically designed to meet the needs of residents and fellows. A lecture miniseries was given by COL Jenice Longfield, LTC Michael Morris, and Ms Robbie Fuqua, BAMC Clinical Investigation and by COL J Michael Lamiell, Clinical Investigation Regulatory Office in the AMEDD Center & School. Residents subsequently attended one of five small group workshops led by department research coordinators and other BAMC/WHMC staff. This allowed residents to discuss research ideas with staff in their chosen specialty. A major objective of the day was to assist residents at an early stage in the development of their projects. No one left empty handed! Residents received numerous handouts on research ethics, protocol writing, informed consent templates, gift and grant rules, IRB meeting dates, and helpful web sites for research, statistics, and research federal regulations. A big thank you for all the behind the scenes direction of events goes to MAJ Sue Baum, Assistant Chief of the Research Consultation Service, Dept of Clinical Investigation. Cookies and tours were offered in the afternoon at the Research Building for those attendees wanting a first hand look at the basic science lab and the research vivarium. Thanks to everyone who helped put this program together and to all the departments who supported the retreat by freeing up residents so they could attend.

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MAJ Sue Baum and MS Robbie Fuqua help attendees check in at Retreat



FROM THE LAB ANIMAL MEDICINE SVC
LTC Richard A. Harris, VC

The Benefits of AAALAC Accreditation for BAMC

At a yet undetermined date between January and March 2003, BAMC will go through the rigors of a re-accreditation inspection by a team from the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. This inspection is conducted very similar to the JCAHO survey. BAMC has maintained full AAALAC accreditation since 1994. Although AAALAC accreditation is voluntary, the DOD has mandated that all laboratory animal care and use programs will be fully accredited.

Many programs and certifications, such as ISO 9000, exist to help meet and exhibit quality goals. In the scientific community, AAALAC International accreditation shows that an institution is serious about setting, achieving, and maintaining high standards for animal care and use in science. More than 600 institutions in 11 countries have earned AAALAC accreditation, making it a symbol of excellence recognized around the world.

For the investigator contemplating a research study with animals, AAALAC accreditation will provide some very meaningful benefits:

1) IT PROMOTES SCIENTIFIC VALIDITY.

When scientific research involves animals, reliable results depend on superior animal care and use programs. AAALAC International accreditation engages scientists, managers and administrators in an independent, rigorous assessment of their institution's animal program—an assessment that ultimately results in better research practices and outcomes.

2) IT DEMONSTRATES ACCOUNTABILITY.

Today, organizations are held to very high levels of accountability especially by the general public.

Although animal research is a controversial issue for some, most people support biomedical research if it's conducted in a humane manner. Accreditation through AAALAC International is voluntary, and demonstrates a willingness to go above and beyond the minimums required by law. It tells the public that the institution is committed to the responsible use and care of animals in science.

3) IT PROVIDES A CONFIDENTIAL PEER REVIEW.

Accreditation first requires an institution to perform its own self-evaluation (an extremely valuable exercise for management). Next, a team of highly qualified professionals provides a confidential, on-site evaluation of the institution's animal care and use program. The independent review assures management that a research program is applying the standards it promises.

4) IT AIDS IN SECURING FUNDING SOURCES.

Many private biomedical organizations, including the American Heart Association and the Cystic Fibrosis Foundation, strongly recommend that grantees be supported by animal programs with AAALAC International accreditation. Besides, BAMC and other DOD agencies such as NIH, NASA, Veterans Affairs and the National Science Foundation, regard AAALAC accreditation as evidence of a commitment to program excellence. The bottom line: private and public funding sources view AAALAC International accreditation as assurance that animal use will be justified and humane, and that appropriate regulations and policies will be followed.

5) FOR MANY INSTITUTIONS, IT IS A RECRUITING TOOL.

AAALAC accredited institutions can use their accreditation as a recruiting tool to attract the best and brightest researchers and professors. Talented professionals look for high-quality programs, and accreditation assures potential employees that the institution is dedicated to achieving the highest standards for animal care and use.

The Laboratory Animal Medicine Service in the Department of Clinical Investigation and the Institution Animal Care and Use Committee exist to maintain the high standards of AAALAC accreditation. All residents and professional staff, contemplating a study involving animals, are strongly encouraged to consult with LTC Richard A. Harris, Chief, Laboratory Animal Medicine Service early in the process for assistance in animal model selection, protocol preparation, and resource requirements and allocation. LTC Harris may be contacted by e-mail on Microsoft Outlook or at 916-1264.



From the Laboratory Science Service
Gerald A. Merrill, Ph.D.

DCI has recently purchased a new instrument, the Luminex 100, in keeping with our commitment to provide a “state of the art” laboratory. The Luminex 100 is produced by a biotechnology company in Austin, TX, which was founded to develop improved *in vitro* diagnostics (IVDs). IVDs are being developed and improved to aid in the detection and treatment of diseases such as cancer, autoimmune disorders, allergies, and cardiovascular disease. Many of the existing IVDs are sensitive and adequately fill the need for which they were developed. Unfortunately, many of these IVDs utilize “one of a kind” technologies and are thus specific for use in analysis of a single sample type or analyte and can be very costly. A major objective of the immunology and biochemistry sections of DCI is to be able to detect and quantify macromolecules of interest to the military medical family at BAMC. The nature of these “samples” for analysis varies greatly with the interests of the investigators and includes toxins, DNA or RNA, biological agents (bacteria, viruses, protozoa etc), enzymes, hormones and many other complex molecules. The goal of DCI was to develop or modify assays for many analytes utilizing instrumentation and technologies that would allow for multiple uses at a minimal cost. The Luminex 100 is proving to be a wise investment for attaining that goal providing a format for performing simultaneous quantification of multiple analytes in a minimal sample volume.

The Luminex system incorporates three mature, well-developed technologies: bioassays, microspheres, and flow cytometry with use of their novel software and proprietary microsphere beads. The heart of the system is their proprietary beads. Each bead has a 5.6µm diameter with one of several functional chemistry modifications on their surfaces for covalent attachments of capture compounds. Each bead has two proprietary dyes incorporated into it; one that fluoresces with red laser excitation in the far-red wavelengths and the other which fluoresces in the infrared wavelengths when excited with the same red HeNe laser. This laser is the classification laser, because based on the ratio of the two dyes (and thus the ratio of the red to infra-red fluorescence) each bead

can be classified as a member of one of up to 100 bead sets as it passes through the laser beam of the modified cell sorter. Fig 1 shows how the classification dye ratios identify select bead sets.

Figure 1: Bead Classification by IR-Red Dye Ratio

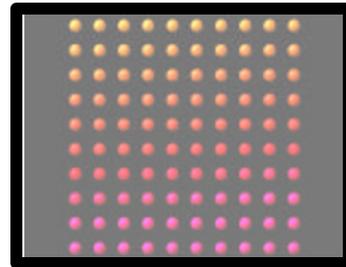
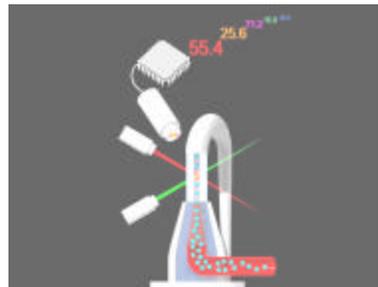
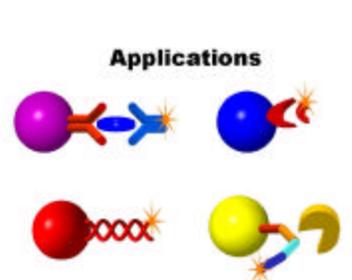


Figure 2: Red laser for Bead Set Classification
Green Laser for Quantification of Assay



The red laser classifies each bead as a member of a unique bead set. Each bead set can be set up to represent a different assay. Quantification of each independent assay is based on excitation of fluorescent compounds with a second (green) laser, which excites at 532 nm. The amount of fluorophore that is excited by the green laser is dependent on the concentration of the assayed material that is present in the sample. Figure 2 shows that as each bead passes through the flow cytometry path, it is evaluated with both a red (bead classification) and a green (assay quantification) laser.

Figure 3: Luminex Assay Formats



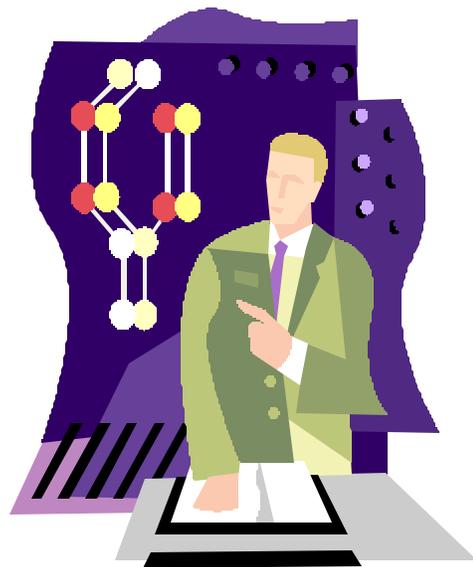
Various formats are available for assay development. These formats, shown in Figure 3, include antigen capture immunoassays (top left), indirect competition assays (top right), DNA/RNA hybridization assays (bottom left), and enzymatic cleavage assays (bottom right). Assays of similar formats can be conducted under the same assay conditions, and thus, simultaneously in a common well of a microtiter plate. Assays that are multiplexed must be evaluated to demonstrate there are no interferences from analytes and reagents from other assays in the same well. Sample volumes can be as little as 5 μ l. As each bead passes through the laser beams independently (there is a size discriminator to prevent analysis of two beads simultaneously), each individual bead is an assay replicate. Typically, 50 – 100 beads of each type are counted from every well of a microtiter plate. With replicates of greater than 50 on every sample, the statistical confidence in assay results is very high. As many as 1.3 million attachment sites are available on each bead, allowing for a large dynamic ranges for quantification of each analyte.

It is the simultaneous multiplexing of assays that allows the Luminex 100 to be used for high throughput screening. The value of high throughput screening with good reproducibility and accuracy was demonstrated by the logistical burden of screening the many environmental and clinical samples generated as a result of the anthrax bioterrorist activity of last year. DCI is currently developing an enzymatic assay in the Luminex format to detect the presence of the lethal factor (a protein subunit of the lethal toxin of *Bacillus anthracis*) to aid in mass screening of samples for anthrax contamination. Our goal is to set up a panel to screen for multiple bioterrorist/BW agents simultaneously. The panel will include other enzymatic cleavage toxins such as botulism neurotoxin A and E, where identification of the toxin would be based on cleavage of unique peptide sequences which could be alleviated by use of specific neutralization antibodies. Multiplex screening assays have been shown to be very cost effective both in terms of time and reagents.

There are currently several multiplex assays commercially available for use on the Luminex. We have been assisting investigators at ISR using commercial kits employing the Luminex technology for rapid analysis of cell culture medium for the presence of 10 cytokines potentially secreted from cultured cells treated with endotoxin. Each cytokine analyzed previously required an independent assay to be performed, each requiring several hundred microliters of sample and the time to perform 10 separate assays. Each cytokine assay had a cost of \$300-\$500, thus making such studies cost prohibitive.

Currently, all 10 assays are performed in 1 day, on a total of less than 250 μ l of sample, and at an affordable price.

There have been numerous applications which have shown the screening power of the Luminex system, including a simultaneous multiplex assay for 16 different grass allergens, panels for 5 infectious pathogens, panels for epitope mapping using overlapping peptides, and DNA hybridization assays for 64 unique DNA sequences simultaneously. We are excited about the potential applications that the purchase of the Luminex 100 system has made possible to BAMC. We are interested in both support of research using commercially prepared kits and in developing “in-house” multiplex assay systems to support research where commercial kits have not yet been developed. To discuss any potential assay development or to further discuss the capabilities of the Luminex system, please contact Dr. Gerald Merrill, DCI Research Immunologist at (210) 916-1353 or the DCI Lab Director, CPT Raven Reitstetter, at (210)916-0613.





CLINICAL INVESTIGATOR'S CORNER

by **John A. Ward, Ph.D.**
with the assistance of **George Vaughan, M.D.**

Recently, I received a pilot study for a power analysis. I don't do power analyses on pilot studies because they are only performed to determine if it is worthwhile to design a complete study. The principle investigator was Dr. Bradley in Radiology. He wanted to determine the feasibility of studying the sensitivity and specificity of an agent for the diagnosis of a disease. The gold standard had a sensitivity of 96% and a specificity of 98%. He planned to estimate the sensitivity of the agent with 5 subjects who had a positive diagnosis with the gold standard. He would plan further studies if at least 4 subjects were positive with the agent. How good is that as a pilot study? For simplicity, let us assume that the sensitivity of the gold standard is 100%, so that all 5 subjects are true positives. The probability of each combination of 2 things (positive, negative) taken 5 at a time can be determined by binomial expansion:

$$(p + q)^5 = p^5 + 5 p^4 q + 10 p^3 q^2 + 10 p^2 q^3 + 5 p q^4 + q^5$$

where p = sensitivity of the test and
 $q = 1 - p$ = probability of a false negative.

The equation was used to prepare the table below. Column 1 is the true sensitivity of the test. Columns 2 through 7 are the probabilities of each combination of 2 things taken 5 at a time. Column 8 is the probability of 4 or more positive tests out of 5. Column 9 is the probability of 3 or fewer positive tests out of 5.

Sensitivity	p (5+,0-)	p (4+,1-)	p (3+,2-)	p (2+,3-)	p (1+,4-)	p (0+,5-)	p>= 4/5	p<= 3/5
0.990	0.951	0.048	0.001	0.000	0.000	0.000	0.999	0.001
0.950	0.774	0.204	0.021	0.001	0.000	0.000	0.977	0.023
0.900	0.590	0.328	0.073	0.008	0.000	0.000	0.919	0.081
0.850	0.444	0.392	0.138	0.024	0.002	0.000	0.835	0.165
0.800	0.328	0.410	0.205	0.051	0.006	0.000	0.737	0.263
0.750	0.237	0.396	0.264	0.088	0.015	0.001	0.633	0.367
0.700	0.168	0.360	0.309	0.132	0.028	0.002	0.528	0.472
0.650	0.116	0.312	0.336	0.181	0.049	0.005	0.428	0.572
0.600	0.078	0.259	0.346	0.230	0.077	0.010	0.337	0.663
0.550	0.050	0.206	0.337	0.276	0.113	0.018	0.256	0.744
0.500	0.031	0.156	0.313	0.313	0.156	0.031	0.188	0.813

From the table, it can be seen that if the true sensitivity of the test is 0.950, then $p \leq 0.023$ that the number of positive tests will be ≤ 3 . The table can be expanded to 3 decimal places to show that if the true sensitivity of the test is 0.924, then $p \leq 0.049$ that the number of positive tests will be ≤ 3 . Beyond that point, the investigator will reject the hypothesis that the sensitivity of the test is 0.924 or better.

Sensitivity	p (5+,0-)	p (4+,0-)	p (3+,2-)	p (2+,3-)	p (1+,4-)	p (0+,5-)	p>= 4/5	p<= 3/5
0.924	0.674	0.277	0.046	0.004	0.000	0.000	0.951	0.049

I took all of this to Dr. George Vaughan in ISR. He proceeded to give me a lesson in Bayesian statistics. He said, "John, your approach, involving assumptions of several possible true sensitivities and then finding the probabilities of various possible observed sample counts of positive tests / number tested (X/N, the sample sensitivity), really only tells you what you might expect in the sample, if the true sensitivity is such and such. It would be more direct to ask what might be the range of true sensitivity, if the sample sensitivity were such and such. That is, if the sample sensitivity

that the investigator might observe were, say 4/5 (or any of the possibilities, 0/5, 1/5, 2/5, 3/5, 4/5, or 5/5), then for each sample possibility, what would be the 95% confidence limits within which the true sensitivity would reside? This requires a Bayesian approach (with a flat prior to avoid bias).”

Wondering how George managed to speak in parentheses, I told him, “I can’t find the 95% confidence interval because the method I use requires a much larger sample.”

He said, “Regardless of the sample size, you can produce an exact confidence interval on the continuous curve of true sensitivity versus the binomial probability distribution function for it, and then by integrating the function to obtain the isodense confidence limits (dropping a line parallel to the x axis until intersections with the curve define points outside of which the tail areas below the curve sum to 5%). In one direction, going from true to sample, $\Pr(\text{sample sens} \mid \text{true sens})$ is a discontinuous curve. But this only represents one side of the binomial coin. That is, in the other direction, going from sample to true, $\Pr(\text{true sens} \mid \text{sample sens})$ is a continuous curve.”

“How do you insert the ‘given that’ symbols?” I asked.

“Like this,” he said and traced a vertical line in the air with his index finger, “|,” and uttered a “pwitt!” in the manner of Victor Borge. He continued, “Taking advantage of this latter relationship, we see that if the investigator finds 4/5 (80%) positive as the sample sensitivity, the true sensitivity could be as low as 41% or as high as 98%, in terms of the 95% confidence limits. Thus, his requirement for a sensitivity as good as that of the gold standard (95%) would be within that range, and it would be reasonable to proceed beyond the pilot phase. On the other hand, if his sample sensitivity is observed to be 3/5 (60%), then the 95% confidence limits for the true sensitivity would be 24% and 90%, making it unlikely that the test under consideration has a 95% sensitivity that characterizes the gold standard. **Therefore, the pilot study requirement of at least 4 positive tests out of 5 subjects best discriminates the potential for the test to have a sensitivity as high as that of the gold standard.** But an estimate of the sensitivity of the new test would then require

larger numbers in the follow-on study.” He underlined and boldfaced his conclusion by sweeping his finger under those lines like the sword of Zorro.



Bulletin Board



CONGRATULATIONS!!!

The 2002 Excellence in Research Award was recently bestowed upon LTC Timothy Flynn, PT, PhD, OCS, FAAOMPT and colleagues at the Annual Conference of the American Academy of Orthopaedic Manual Physical Therapists (AAOMPT) in Orlando, Florida. The team of physical therapists included Flynn, Dr. Julie Fritz, CPT Julie Whitman, LTC Rob Wainner, LCDR Jake Magel, MAJ Dan Rendeiro, CDR Barb Butler, MAJ Matt Garber, and COL Steve Allison. The AAOMPT is an organization that promotes excellence in orthopedic manual physical therapy practice, education, and research. The prestigious award recognized a collaborative project of the U.S. Army-Baylor Graduate and Post-Professional Doctoral Programs in Physical Therapy, Brooke Army Medical Center, Wilford Hall Air Force Medical Center, and the University of Pittsburgh, entitled "A Clinical Prediction Rule for Classifying Patients with Low Back Pain Who Demonstrate Short Term Improvement with Spinal Manipulation." The study examined 75 patients with moderate to severe low back pain, and identified those individuals that responded favorably to a standardized spinal manipulation treatment program. The researchers demonstrated that the presence of certain factors in the history and physical examination could increase the probability of success with spinal manipulation from 45% to 95%, thus allowing patients likely to respond dramatically to be clearly identified prior to treatment.

This research will be published in the December 15th issue of *Spine*. Recognized internationally as the leading journal in its field, *Spine* is an international, peer-reviewed, bi-weekly periodical that is the leading subspecialty journal for the treatment of spinal disorders. LTC Flynn is the Program Director and LTC Rob Wainner is the Research Director of the nationally ranked U.S. Army-Baylor University Graduate Program in Physical Therapy at the Academy of Health Sciences; MAJ Rendeiro is the Program Director of the U.S. Army-Baylor Postprofessional Doctoral Program in Orthopaedic & Manual Physical Therapy at Brooke Army Medical Center, Fort Sam Houston, TX.

**The deadline for the
2003 Commanders' Research
Awards Competition
is**

7 April 2003

See your Program Director for submission guidelines. The competition is open to San Antonio Uniformed Services Health Education Consortium (SAUSHEC) Medical Corps housestaff, Oral Surgery and University of Texas Health Science Center-San Antonio (UTHSCSA) integrated program physician graduates.

SAUSHEC Program Directors are encouraged to assist all SAUSHEC Medical Corps housestaff, Oral Surgery, and UTHSCSA integrated program physician graduates in making timely progress on their research projects.

From our readers...

Readers are invited to send comments or questions about items printed in *Research Review*. Requests for research information and topics for future publications also invited. Send to one of our mailing addresses on the front page. Responses from readers will be published in this column unless requested otherwise.



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