

The Ohio State University Medical Center  
Dorothy M. Davis Heart and Lung Research Institute

**Core Lab Policies and Procedures**

This document is intended to provide users of the Davis Heart and Lung Research Institute (DHLRI) Core Laboratories with detailed information about the use of these facilities

The Davis Heart and Lung Research Institute provides research services in the following core laboratories:

- Flow Cytometry
- Microscopy
- Genetic and Microarray
- Atomic Force Microscopy (AFM)
- Integrative Cardiovascular Physiology (ICP)
- Electron Paramagnetic Resonance (EPR)/ Nuclear Magnetic Resonance (NMR)

As part of the DHLRI, the core laboratories provide specialized equipment and services not routinely available in individual laboratories. The core laboratories are staffed by experienced specialists who provide training and technical assistance for the operation of equipment available in each lab. This document provides a general description of the function and major equipment provided in each of the core laboratories. In addition, it lays out the policies that govern the operation and use of the core facilities. Investigators and their staff who use the facilities will be expected to read and observe the following operating procedures. Continued access to these resources will be contingent upon appropriate interaction with staff, and responsible use of all equipment as well as payment of charges incurred.

**Core Operations Committee**

The Core Operations Committee was formed by the institute director with the charge of providing oversight, direction and support for the core facilities of the institute.

**Core Operations Committee Mission Statement**

The Davis Heart and Lung Research Institute is committed to providing its investigators with access to state-of-the-art technologies in well-organized and maintained core facilities to optimize their investigations of cardiovascular and pulmonary diseases. The Core Operations Committee will provide oversight, direction and support for the core facilities of the Institute. Functions of the Core Operations Committee will include:

- Developing long-range planning for the cores
- Serving as a resource to advise the lab directors
- Monitoring customer satisfaction with the cores
- Providing oversight of core budgets
- Making decisions about new equipment purchases, management of existing equipment, and construction requests
- Providing assistance and oversight in the recruitment and interviewing of personnel for the Cores
- Reviewing the performance of core directors, managers and personnel.

### **Core Laboratory Directors**

A manager and a director will oversee the operation of each core laboratory. The responsibilities of the core laboratory director will be as follows:

- Serve as liaison between the manager and the DHLRI Director
- Provide scientific advice to the manager and DHLRI investigators
- Serve as liaison between managers and investigators as needed
- Oversee effective operation of the core laboratory
- Advise about pricing structure and profitability
- Provide input on latest equipment and software available to enhance laboratory operations
- Assist manager in developing and implementing laboratory policy and procedures
- Serve on the DHLRI Operations Group Committee

It is important to note that each Core Laboratory Director will be expected to pay the same charges for use of core equipment as other investigators. Their scheduled time on the equipment will also follow established scheduling procedures that apply to all investigators.

### **Core Laboratory Managers**

The managers will have the following general responsibilities with regard to their laboratories:

- Oversee daily operation of the core laboratory
- Schedule use of core laboratories
- Monitor equipment maintenance
- Provide input on latest equipment and software available to enhance laboratory operations
- Troubleshoot equipment problems
- Assist principal investigators in study design
- Assure compliance with applicable safety regulations
- Determine usage charges and initiating monthly billing to projects
- Train staff on use of core equipment
- Assist in grant application and design
- Serve on the DHLRI Operations Group Committee

### **Core Laboratory Users**

There are three categories of users for the core laboratories. They are DHLRI members, OSU non-members, and non-OSU users. The DHLRI Executive Committee is responsible for establishing the criteria for membership and for approving individual membership applications (*See the DHLRI website for the current membership policy*). The difference between member and non-member users is reflected in the usage rates charged by the cores, and in the scheduling priorities during peak hours (see next section). Other than these two items, members and non-members are bound by the same operating policies.

## **Core Laboratory Access and Equipment Use**

Prior to using a core lab, the principal investigator must establish an account with the billing office of the DHLRI. This account authorizes the monthly billing of charges generated in one or more core labs. Forms are available on the DHLRI website for establishing the account. A completed and signed form must be submitted by the PI prior to any work being performed in the core labs. In addition, each individual user must submit a completed and signed access registration form, authorizing them to incur charges on behalf of the PI. A separate form must be submitted for each core lab used by each individual.

After submission of all required forms, core managers will determine the level of access for the user based on their equipment needs. Some equipment will require completion of a formal training course before a user can utilize the instrument. Investigators should contact the core manager well in advance of their anticipated needs in order to ensure that they either have the necessary skills, or will be able to obtain the required training prior to their planned use of the equipment. Please note that completed forms must be approved by the core managers prior to any scheduling or actual usage of equipment. Continued use of core facilities requires that the PI maintain an active billing account with the DHLRI billing office.

Where appropriate, 24-hour access to the core via key card will be authorized. When granted, such access is only for the authorized user, and then only for the equipment that the user has been approved to use.

The following points provide important access information:

- Core equipment that is designated only for core operators is off-limits to all users at all times.
- All core access and utilization requires prior authorization by the core manager. Core managers will specify the registration forms and training necessary for users to become authorized. Authorization is specific to equipment and level of operation.
- Access to core facilities is available 24 hours a day for trained and approved users. Off-hour access is by key card only. Key card access to the core labs is approved by core managers. Access is contingent on having active account information on file with the DHLRI business office. Core managers will review usage on an annual basis and cancel inactive accounts.
- In the unlikely event that usage levels dictate, the core manager may limit non-member scheduling of equipment time during regular hours (8:00AM – 5:00PM) for the upcoming week to 40%, with the remainder reserved for priority scheduling by DHLRI members. At the beginning of each week, any unscheduled time for the current week becomes available regardless of membership level.
- Damage to equipment resulting from misuse may result in loss of privileges and payment of repair costs.

## **Core Laboratory Training**

It is the responsibility of the core manager to ensure that all users are properly trained for the equipment they are authorized to use. The training will vary greatly depending upon the complexity of the equipment, and may be as simple as a ten minute demonstration by the core manager, or it may involve several hours of lecture and hands-on evaluation by the manager. Some sessions may involve a training charge, so be sure to discuss the training with the manager before signing up for it. Training in another facility or familiarity with a similar piece of equipment does not negate the requirement to prove competence to the core manager's satisfaction. After discussion of previous experience with the manager, it may still be required to attend an abbreviated (or possibly a full) training session. Managers will maintain records of users who have undergone formal training. Principal investigators can contact the manager for information on their staff's training and usage of core facilities.

## **Core Laboratory Charges**

Each of the core laboratories will be a cost-recovery center within the Davis Heart and Lung Research Institute. Managers will maintain a price list for the services of their laboratories. There are different usage charges for DHLRI members and non-members, as well as for regular business hours and after-hours use. For the purposes of rate determinations, regular hours for all core labs are defined as 8:00 AM to 5:00 PM, Monday through Friday, University holidays excluded. Note that the hours of manager availability may be different from the regular hours and may vary from core to core. Contact the core managers to determine their hours of availability. Charges for services are subject to change without advance notice. Any changes in pricing will be posted to the DHLRI website along with their effective date. Users are encouraged to consult the website for the current price list before initiating any new studies. Charges will be billed monthly to the account number on file in our business office, and investigators will receive a summary of charges incurred for the month. Scheduling of core equipment or core personnel is considered a commitment of resources and, therefore, a billable event, whether or not the user follows through with the reservation. Users are encouraged to carefully read the following section on the scheduling policy for the DHLRI core labs. Feel free to contact any core manager if there are questions regarding any of the rules.

## **Core Laboratory Scheduling**

Users can check availability and schedule time for core equipment by accessing the online calendars located at the DHLRI website. The web calendar may also be used to request assistance time by the core personnel. However, all assisted appointments are only tentative until confirmed by the core person involved. Checking on the availability of the core person before scheduling is generally recommended in order to expedite the process. Scheduling of off-hours assistance is always at the discretion of the core personnel and will carry a surcharge beyond the normal charges. To schedule equipment, go to the individual core lab web page and select the appropriate calendar based on the equipment/software you wish to use. If requesting core assistance, please refer to the individual core lab page for general hours of operation. For non-assisted operation, authorized personnel will have 24-hour access. The scheduling policy of the DHLRI core labs is designed to encourage the maximum availability of the facilities to all users. With this in mind, repeated over-booking or no-shows for scheduled time slots will be considered billable usage of the facilities. Users are expected to make reasonable estimates when booking time. If the core manager observes a pattern of scheduling excess or unused time, the user will be notified, after which they will be liable for all future scheduled time whether used or not. Core personnel are aware of the unpredictability of experimental setups and that

planned experiments may need to be cancelled. Communicating problems to the core managers as soon as possible is the best way to avoid default charges. There will be no charge for cancellation of scheduled time if the core manager is notified at least 24 hours prior to the scheduled time. Charges for cancellations less than 24 hours in advance will be determined by the core manager based on the current demand for the equipment and the frequency of past cancellations by the user. A no-show with no cancellation call; however, can result in the entire amount of scheduled time, as well as any set up time, being charged to the investigator's account, with no consideration given to the user's history or equipment demand. If a user is late for a scheduled appointment during regular core hours, they must contact the core manager no later than 30 minutes after the scheduled start time to request the time slot be held. Note that the user can only guarantee their original time block will be held. Extending the time block to compensate for missed time will depend upon availability of the equipment and the manager. In either case, the user is responsible for the originally scheduled time slot. Scheduled time slots not claimed after 30 minutes become available for rescheduling to other users at the discretion of the core manager. Any unused time may be billed to the originally scheduled user. In the event that equipment in a core lab is not functioning properly, affected customers will be notified as soon as possible. To expedite this notification, users should make sure their contact information filed with the core is up-to-date and complete. The schedule will be adjusted to accommodate the time necessary for servicing the equipment. Once the equipment has been repaired, users bumped by the downed equipment will be given the first opportunity at scheduling the initial open time slots. To minimize the amount of equipment downtime, users should always report any problems with the equipment even if they were able to complete their work.

### **Core Laboratory Equipment Damage**

Users who demonstrate misuse or abuse of equipment, scheduling, or core policies may have their access to all core equipment suspended. Core directors will be responsible for issuing any warning or suspension of privileges. If warranted, the core manager may require the offending user to repeat previous training sessions (including payment of any training charges) before reauthorizing the user. Please note that use of equipment for which you have not received prior approval is considered misuse, regardless of whether or not actual damage or incorrect operation of the instrument has occurred. In addition, it is considered misuse for any user to give access (either by key card or passwords) to an unauthorized person. All damage determined to be the result of misuse or neglect will require payment of needed repairs by the authorizing PI. The core directors will grant access to users whose privileges have been suspended only after the period of suspension has been completed and any damages have been reimbursed. A second offense will require a review meeting of the DHLRI Core Managers/Directors Committee to approve reinstatement of laboratory usage.

### **Core Laboratory Computer Usage**

All users of the core laboratories computers will follow the ***Policy on Responsible Use of University Computing Resources*** located at <http://www.oit.osu.edu/links.html>). The use of some core computers may require a user account and password. These accounts will be issued by the core manager. The accounts may be on a per use or per lab basis, as determined by the policy of the core lab. Because you have the password to sign on to a piece of equipment does not mean that you also have the authorization to use it. Also, you cannot "loan" your authorization to someone else.

The core labs will provide disk space on workstations for data acquisition and processing, but not for data storage. The amount of space available will be determined by the core manager, based on the hardware available, and the number of users. Data files should be moved to the appropriate long-term storage (network shared drives or removable disks) upon completion of acquisition and processing.

All users are responsible for archiving data files they deem important to preserve. These may include raw data, intermediate or final processed files and images. Methods available for archiving will depend upon the user and workstation, but may include transfer to CDs, Zip disks, or the DHLRI servers. The Core Computer Services (CCS) provides directory space (10 GBytes) for DHLRI principal investigators. CCS performs daily backups of all data stored on the central servers, thereby allowing investigators to meet their archiving needs by simply transferring data files to research folders on the servers.

### **Electron Paramagnetic Resonance (EPR) Core/ Nuclear Magnetic Resonance (NMR)**

- Location: EPR- 134, 130, 144, & 150, TMRF, 635 DHLRI, 0090E, & 365 BRT  
NMR- 190 TMRF & 0084 BRT
- Hours of operation: 9:00 AM - 5:00 PM
- Director: Periannan Kuppusamy, PhD
- Manager: Nancy Trigg
- Phone: (614) 292-8998 or (614) 292-9033
- E-mail: hlri-epr@osumc.edu
- Fax: (614) 292-8778

The Biomedical EPR Spectroscopy and Imaging Core offers cutting edge magnetic resonance technology for detection, quantitation, and visualization of free radicals in biological systems. Electron paramagnetic resonance (EPR), also termed electron spin resonance (ESR) spectroscopy, is a magnetic resonance technique capable of directly detecting free radicals and paramagnetic species. In addition to quantitative spectroscopic measurements, imaging of free radicals can be performed using this technique. The method is routinely used to measure free radicals such as superoxide, hydroxyl and nitric oxide in chemical/biochemical/biological systems. EPR also enables measurement and imaging of physiologically pertinent tissue parameters (*functional imaging*) such as tissue perfusion, oxygenation, metabolism, redox state, viability, pH, etc. using appropriate spin probes.

The EPR Core has an array of multi-frequency EPR spectrometers, and resonator designs, capable of measurement of samples ranging from molecules to man. *In vitro* measurements of free radicals can be made in aqueous solutions, or tissues, in as small as 50 uL volume. Short-lived free radicals of “reactive oxygen species” can be determined using stabilizer molecules called spin traps. Free radical measurements and imaging can also be performed in intact tissue, organs and in whole-bodies of small animals (mice and rats) using low-frequency (300 MHz – 1200 MHz) spectrometers.

The Core also has the capability for high-resolution (0.1 mmHg) oxygen measurements, *in vivo*. Repeated (days to weeks) measurements can be made from a specific site of the animal/organ/tissue using implantable micro/nano-particulate oximetry probes.

The following resources are currently available:

- Three Bruker ER-300 X-band (9.7 GHz) Spectrometers equipped with variable temperature assembly, including liquid nitrogen capability.
- One Bruker EMX X-band spectrometer with SuperQ resonator and aqua-X cell for high sensitivity measurements from small amounts of samples.
- An L-band (1.2 GHz) *in vivo* EPR spectrometer and imager capable of free radical measurements from tissue, intact organs and small animals.
- A 740 MHz *in vivo* EPR spectrometer and imager capable of free radical measurements from larger (mouse, small rat) biological objects.
- An S-band (3 GHz) imager for topical (surface) measurements.
- A 300 MHz imager for XL (extra-large) objects and a whole-body human imager (MRI/EPRI) are under construction.

The facility has a variety of spin probes including spin traps, oximetry, pH and redox probes for biological applications, and a team of experts to help you with your applications.

### **University Cell Analysis and Sorting Core (formerly Flow Cytometry)**

- Location: DHLRI, Rm. 420, 424, 426
- Hours of operation: 8:00 AM - 5:00 PM
- Director: Jeffrey Chalmers, Ph.D.
- Manager: Bryan McElwain
- Phone: (614) 292-FLOW (614/292-3569)
- E-mail: hlri-flow@osumc.edu
- Fax: (614) 247-7799

The University Cell Analysis and Sorting Core provides analysis and/or physical sorting of cells in liquid suspension according to the expression of selected cell properties. Analysis possibilities include cell surface phenotype markers (anti-CD3, CD4, etc.), gene expression reporters (GFP, YFP, etc.), cell cycle analysis, cytokine production, apoptosis, cell viability, rare cell detection, and calcium metabolism. Consultation in experimental design and review of grant applications and manuscripts is also offered.

The flow cytometry core has 3 instruments:

- **Becton-Dickinson FACS Calibur:** equipped with 4 PMTs allowing for four-color analysis using a 488nm air-cooled argon and a 633nm helium-neon laser as excitation wavelengths. The sort option is also available on this FACS Calibur flow cytometer.
- **Becton-Dickinson FACS VantageSE:** equipped with 5 PMTs allowing for five-color analysis and sorting using air-cooled 488nm argon and 633nm helium-neon lasers and a water-cooled argon UV laser as excitation sources. In addition, this instrument will have a turbo-sort option (25,000 cells/sec target capture) and a CLONECYT single-cell or multicell deposition system for microtiter plates or microscope slides.
- **Becton-Dickinson FACS Aria:** equipped with 8 PMTs allowing for seven-color analysis and sorting using 488nm and a 633nm lasers. The Aria is the first high-speed sorter with a fixed-alignment flow cell.

Software such as Win List, WinMDI, Modfit, Multicycle and Cellquest Pro is available to investigators for off-line analysis. Imaging output software such as Avid Video, Photoshop, Pagemaker and Microsoft Office is available on either PC or Macintosh systems.

### **Microscopy**

- Location: 480 DHLRI
- Hours of operation: 7:00 AM – 4:00 PM
- Director: Sandor Gyorke, Ph.D.
- Manager: Alan Bakaletz
- Phone: (614) 247-7655; 247-7688
- E-mail: hlri-micro@osumc.edu
- Fax: (614) 247-7799

The role of the Microscopy Core is to provide microscopy workstations capable of advanced imaging techniques beyond that of the standard laboratory microscopy.

- The **Nikon Eclipse 800** is a wide-field, transmitted-light and epi-fluorescence microscope. It is equipped with high-quality objectives from 1x to 100x. Its differential interference contrast optics allow detailed visualization of many unstained specimens. For fluorescence, filter sets are available for DAPI, FITC, and Rhodamine type probes. Image acquisition may be done in two ways: 1) traditional recording on film, using the Nikon FDX-35 camera; 2) digital recording, using the Photometrics SenSys CCD digital camera and the MetaMorph 6.0 software. The MetaMorph software provides advanced imaging capabilities in the areas of 1) image acquisition – multicolor overlays, time lapse; 2) image processing – filtering to alter contrast, edges, detail, and object separation; and 3) image analysis – a variety of morphometric techniques for measuring intensities and spatial dimensions as well as object localization and counting.
- The core also has a confocal, laser scanning microscope, (**Zeiss LSM510**) This microscope has three visible range lasers, producing six laser lines (458, 477, 488, 514, 543, and 633 nm) for fluorescent probe excitation. The microscope has a variety of short, long and band pass filters permitting the visualization of any of the standard visible range fluorescent dyes. In addition, this microscope has an infra-red laser, tunable through the range of about 720 – 900 nm. This laser is useful in live-cell work, allowing the two-photon excitation of many of the visible range dyes.

### **Genetics/Microarray**

- Location: 664 DHLRI
- Hours of operation: 9:30 AM - 5:30 PM
- Director: Chandan K. Sen, Ph.D.
- Manager: Leni Moldovan, PhD
- Phone: (614) 247-7484
- E-mail: hlri-gene@osumc.edu
- Fax: (614) 247-7799
- Equipment: ABI PRISM 7700 Sequence Detector  
Affymetrix GeneChip Microarray System

The genetic/microarray laboratory will utilize the newest technology and protocols for complete genetic analysis. The genetic component is an integral step involved in understanding heart and lung disease. It is vital that such a core laboratory be accessible to investigators in this field, especially in the advent of the Human Genome Project. Understanding the genetic background of heart and lung disease is essential in developing clinical applications, and pursuing the field of pharmacogenetics.

The Genetics/Microarray Core has the following equipment and resources:

- **GeneChip Microarray System** (Affymetrix, Inc., Santa Clara, CA)  
This system includes the GeneChip Hybridization Oven 640, the GeneChip Fluidics Station 400, the GeneArray Scanner and a computer workstation loaded with Affymetrix Microarray Suite for instrument operation and data analysis. This system is capable of a broad scale gene expression profiling and SNP detection.
- **ABI PRISM 7700 Sequence Detection System** (Applied Biosystems, Foster City, CA)  
This is a fully integrated system for real-time detection of PCR. The system includes a built-in thermal cycler, a laser to induce fluorescence, CCD (charge-coupled device) detector, and Sequence Detection System Software Version 1.7. This system can provide Quantitative Gene Expression using Taq-Man real-time PCR and SNP detection: TaqMan allelic discrimination assay.

Services:

- Gene Chip Microarray for gene expression
- Gene Chip Microarray for SNP detection
- Real-time PCR (users operation, training provided)
- SNP detection using Allelic Discrimination technique
- Consulting for study design, sample preparation, and large sample preparation.
- Training/seminars for Microarray and real-time PCR techniques
- Reagents/supplies for Microarray and Applied Biosystems for lower prices on chips reagents and supplies.

Training sessions for Microarray and real-time PCR are provided every month. The Microarray array training is on the 2<sup>nd</sup> Wednesday of the month from 2:00 PM to 4:00 PM. The real-time PCR training is offered on the 3<sup>rd</sup> Wednesday from 2:00 PM to 4:00 PM and is limited to six people in each session. RSVP is required to [hlri-gene@medctr.osu.edu](mailto:hlri-gene@medctr.osu.edu). Training is mandatory for new users, and there is a charge of \$100 for members and \$150 for non-members for a two-hour training session.