

DIABETES ENDOCRINOLOGY RESEARCH CENTER
Application for Affiliate Status

Project Title: _____

Principal Investigator: _____ Degrees: _____

Academic Title: _____

Department: _____

Mail Stop: _____ Phone: _____

Email _____

Co-investigator(s)/Collaborators(s):

Name	Academic Title	Departmental Affiliations
_____	_____	_____
_____	_____	_____
_____	_____	_____

Current Research Funding:

Agency	Agency #	Principal Investigator	Current Amount
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Cores to be Utilized (check all that are applicable):

- | | |
|--|--|
| <input type="checkbox"/> Clinical Research Core | <input type="checkbox"/> Cellular and Molecular Imaging Core |
| <input type="checkbox"/> Mass Spectroscopy Core | <input type="checkbox"/> Cellular Biology Core |
| <input type="checkbox"/> Molecular and Genetics Core | <input type="checkbox"/> Islet Cell and Functional Analysis Core |

BRIEFLY DESCRIBE SERVICES (TYPE AND AMOUNT) TO BE PROVIDED BY EACH CORE CHECKED AS DETERMINED THROUGH DISCUSSION WITH THE CORE DIRECTOR (a list of names and phone numbers is attached):

On the following page please provide a short summary of the proposed research project.

Signature: _____ Date _____

PROJECT SUMMARY

Principal Investigator: _____

Project Title: _____

INSTRUCTIONS FOR SUBMITTING YOUR APPLICATION

Please send one original and 11 copies of your completed application and one copy of your C.V. or NIH Biosketch to Corinne Lovato, DERC Manager at Campus Box 358285 or send to the following mailing address:

**Diabetes Endocrinology Research Center
DVA Puget Sound Health Care System (Box 358285)
1660 S. Columbian Way
Seattle, WA 98108**

If you have any questions regarding the application, please contact Corinne Lovato at 206-764-2692.

TO: Diabetes Endocrinology Research Center Affiliates

FROM: Jerry P. Palmer, M.D.
Director

SUBJECT: Services of the Diabetes Center

The Diabetes Endocrinology Research Center consists of six biomedical research Cores. The enclosed comments describe the services each provides. Affiliates are urged to contact the Core Directors as early as possible in planning future needs. Corinne Lovato is the Center Manager and can answer administrative questions regarding Center operations (764-2692). Any major change in research direction which involves Core use will require formal approval by the Executive Committee. In general, a chargeback will be made for all Research Core uses. New pilot and feasibility initiatives will be funded through an internal and external review system as approved by NIH. If currently awarded funds become available due to the identification of alternate sources of support, a call for pilot and feasibility studies will be made.

Immunoassay services are available to DERC Affiliates at the Northwest Lipid Research Laboratories (NWLRL): For more information and fees, contact Dr. Santica Marcovina, NWLRL Director, at (206) 685-3331. (smm@u.washington.edu)

Please visit our website at <http://depts.washington.edu/diabetes/> for more information, and for our current chargeback rates.

When preparing manuscripts relating to studies that have been aided by the Core facilities, please give credit to the Diabetes Endocrinology Research Center (Grant # 5 P30 DK17047) in the acknowledgement portion of the manuscript and send us a copy of the reprint when it becomes available.

CLINICAL RESEARCH CORE (CRC)

The CRC offers assistance to Diabetes Center Affiliate investigators in several areas. The first is research subject recruitment, in which the Clinical Core provides the investigators with potential volunteers for their research studies. The second service offered by the Clinical Core is obtaining blood, skin, or pathological specimens for investigators. The Clinical Core maintains a computerized registry of patients with diabetes who have expressed an interest in being research subjects. In addition, the Clinical Core can actively recruit highly specialized subjects including matched controls, through contacts with referring physicians and media advertising. Recently, the Clinical Core has established a link with the University of Washington Medical Center's Diabetes Care Center which enables us to provide investigators with volunteers that meet certain metabolic criteria. Lastly, the CRC provides biostatistical support services for DERC Affiliates.

Any investigator who needs research volunteers is requested to contact the Core Director, Dr. Boyko, to discuss the proposal and recruitment requirements. After ensuring that the investigator has appropriate human subjects approval, the Core's research coordinator will recruit subjects matching the investigator's requirements from the DERC Diabetes Registry or other sources of study subjects as appropriate. During our initial contact with potential study subjects, we describe the proposed study in a general sense, ascertain the willingness of the subjects to participate and determine whether they meet the investigator's criteria. Subjects who agree will then be referred to the investigator for complete explanation of the study, and informed consent. Information on each individual's research participation and the volume of blood that is drawn are entered into the Diabetes Registry database. We contact investigators one month after referral of a potential study subject to determine if the referred subject participated in the study.

Investigators who need only specimens for their projects also may contact Dr. Boyko to review their needs. For many studies, the Clinical Core can obtain informed consent, obtain by venipuncture blood samples, process blood samples if necessary (i.e., separate serum, freeze), and deliver the specimen to the investigator's laboratory at a time convenient for the investigator.

Investigators desiring biostatistical services should Contact Dr. Boyko about their needs.

The CRC provides for searching of the computerized registry for suitable research participants and the personnel necessary for recruiting subjects and obtaining specimens. Affiliate investigators may be asked to bear the costs of advertising. The CRC charges investigators \$5.15 for each volunteer referred, \$15.45 for each volunteer referred with cells, and \$10.30 for each research specimen obtained. Investigators are billed monthly for these expenses by journal voucher.

We look forward to actively assisting diabetes investigators and encourage you to discuss your needs with us.

Ed Boyko, M.D.

Core Director

VA Puget Sound Medical Center

Mail Stop S-111

1660 S. Columbian Way

Seattle, WA 98108

(206) 764-2773

University of Washington Box 358280

eboyko@u.washington.edu

CELLULAR AND MOLECULAR IMAGING CORE (CMIC)

The CMIC offers technical help with morphological techniques that Affiliate investigators need for their research projects. These services include:

- **Tissue fixation and processing for light microscopy:** Preparation of fixative solutions, performing vascular perfusions, collecting tissues, and freezing tissues.
- **Embedding and sectioning:** Paraffin embedding of fixed tissues and embedding in OTC compound for cryostat sectioning. Paraffin sectioning as well as cryostat sectioning of frozen tissues. Sections are mounted on slides for staining.
- **Histological and histochemical staining:** Routine methods such as hematoxylin and eosin, and specialized histochemical staining techniques such as thioflavin S and Congo red for islet Amyloid, Oil red O for adipocytes, β -galactosidase for *lacZ* reporter gene expression, and TUNEL for apoptosis.
- **Immunocytochemical staining:** Peroxidase and fluorescent-labeled antibodies and related methods, including multiple labeling applications.
- **In situ hybridization:** mRNA localization using isotopic (film and emulsion autoradiography) and non-isotopic methods and with labeled riboprobes and oligonucleotides, including multiple ISH labeling.
- **Flow cytometry:** The core oversees the operation and maintenance of flow cytometry instruments for DERC investigators and trains users.
- **Digital image acquisition:** Images of cellular and histological preparations by various microscopic optical modalities including confocal microscopy.
- **Technical assistance:** Personnel to perform services and train users in protocols and use of analytical equipment for cellular and molecular imaging.
- **Consultation:** Consulting assistance with protocols and procedures, as well as with design of their studies and interpretation of results. Consultation is a major function of the core and involves collaborative interactions of affiliates with the core directors.

Investigators will be assessed chargeback fees for core services and facilities. Investigators submitting new grant applications should budget funds for CMIC services. Please direct questions about services and chargebacks to a CMIC Director.

Denis Baskin, Ph.D.

Core Director
(206) 768-5222

baskindg@u.washington.edu

James E. Blevins, Ph.D.

Associate Core Director
(206) 277-6774

jblevin@u.washington.edu

VA Puget Sound Medical Center
Mail Stop S-151
1660 S. Columbian Way
Seattle, WA 98108

University of Washington Box 358280 (151)

MASS SPECTROSCOPY CORE (MSC)

The Protein Mass Spectrometry Core of the DERC provides the powerful tools of modern mass spectrometry to DERC investigators to permit structural identification and quantitation of biomolecules involved in the diabetes and its complications. In addition to providing instrumental capabilities, the Protein Mass Spectrometry Core staff provides consultation and collaboration on application of protein mass spectrometry and proteomics to diabetes research, including development of new analytical methods targeted at achieving research objectives of DERC investigators. Services offered range from identification of proteins from in-gel digests using peptide mass fingerprinting to relative quantitation of complex protein mixtures by tandem mass spectrometry using stable isotope labeling techniques (such as ICAT) and includes assistance with grant preparation.

For more information about the MSC and its services, contact Pattie Green, Ph.D., Associate Core Director.

Jay Heinecke, M.D.

Core Director
(206) 543-3158
heinecke@u.washington.edu

Department of Medicine (Box 356426)
University of Washington, Seattle, WA 98195

Pattie Green, Ph.D.

Associate Core Director
(206) 616-4860

psgreen@u.washington.edu

VA Puget Sound Medical Center
Mail Stop S-151
1660 S. Columbian Way
Seattle, WA 98108
University of Washington Box 358280 (151)

CELLULAR BIOLOGY CORE (CBC)

The Cellular Biology Core (CBC) provides the following services:

1. Cells and cell cultures
2. Tissue culture equipment facility
3. Medium and buffer preparation
4. Consultation and technical assistance with experiments

Cells and Cell Cultures

The CBC has repository of cultured cells which includes human skin fibroblasts from about 200 donors (control, NIDDM, IDDM, and fetal), several islet cell lines (HIT, HIP, RIN, alpha-TC, and beta-TC), mouse myeloma cell lines NS-1 and P3X63 Ag8.653 (for hybridoma production), several other mouse cell lines (e.g., 3T3-L1), and several hybridoma cell lines. These cultures have been frozen and stored in liquid nitrogen, and will be provided to Affiliates either frozen or after being reinitiated into culture.

The CBC also will initiate cultures from skin biopsies (fibroblast) and other types of tissues provided to it by Affiliates. Examples include newborn rat pancreata (islet monolayer cultures) and human islet cell tumors. The CBC will immortalize peripheral blood human lymphocytes by E-B virus transformation. Transformed cells may be either stored in the CBC or transferred to the Affiliate. A satellite facility of the CBC will also provide isolated islets (rat, dog, human) and cell sorting (by FACS).

In order to obtain cultures from the CBC, Affiliates should indicate the type and number of cultures desired and a brief summary of the project in which the culture will be used.

Tissue Culture Equipment

The CBC will provide access to the use of some equipment by Affiliates who are using tissue culture in their research. This facility is located at the University of Washington Medical Center and includes biological safety cabinets, tissue culture incubators, phase contrast microscope (with photomicroscopic and fluorescence microscopy capability), and other specialized instruments and equipment.

Affiliates who wish to use this facility should submit a brief summary of their project in which they indicate their equipment needs, kinds of cells being used, approximate scheduling requirements, and list of individuals who will be using the facility. They will be oriented to the use of the equipment before being allowed to work in these areas.

Medium and Buffer Preparation

The CBC will prepare a wide variety of culture media, buffers, and balanced salt solutions. All solutions are sterility-tested prior to release from the facility. Currently, the CBC prepares over 100 liters of solutions each month.

Affiliates who wish to take advantage of this service should submit a brief summary of their project and indicate the type and amounts of solution they require. If this is a "custom" solution, then the directions for preparation should also be submitted. Affiliates must provide the CBC with bottles and the powder base for media, as well as any special additives. The CBC will supply those salts commonly used for constructing buffers and salt solutions.

Consultation and Technical Assistance

Consultation concerning tissue culture is available from the CBC. In addition, the CBC will provide technical assistance to Affiliates in order to allow the running of their experiments in the CBC. These include assistance with the growing of hybridoma cultures provided by Affiliates or from the CBC repository, performance of both static and perfusion incubations, hormone (insulin and glucagon) radioimmunoassays of both incubation media and cell (tissue) extracts, and DNA measurements of cell extracts. When experiments are done in the CBC, data analysis and summarization will also be provided.

The above services are periodically revised and new services added.

Affiliates who wish to use this service should submit a brief summary of their project to one of the Core directors. This will be followed by a meeting with the Director to discuss the goals of the project and possible ways to address them. A research plan will be developed and discussed with the technical staff of the CBC.

In addition to the requirements indicated above for use of these services, the Diabetes Research Center has developed a charge schedule for each of the Cores. This schedule is available from the Administrative Core. Affiliates will also be expected to furnish the CBC with an annual progress report.

Alan Chait, M.D.

Core Director
(206) 543-3158

achait@u.washington.edu

John Oram, Ph.D.

Associate Core Director
(206) 543-3470

joram@u.washington.edu

Department of Medicine (Box 356426)
University of Washington, Seattle, WA 98195
FAX: 685-3781

ISLET CELL AND FUNCTIONAL ANALYSIS CORE (ICFAC)

The Islet Cell and Functional Analysis Core (ICFAC) is a broad-based resource for research involving pancreatic islets. Services include preparing isolated islets from rodents and primates, chronic and acute treatment and culture of isolated islets, and experimental analysis of islets and islet cells in regard to their metabolic, functional and morphological state. In addition, consultation regarding experimental design and data analysis is provided. Classes of studies carried out have involved control of insulin secretion, beta cell death, mitochondrial metabolism, stimulation secretion coupling, beta cell surface monoclonal antibody production, phenotyping genetically altered mice, transplantation studies, and binding and uptake studies. Flow culture combined with sophisticated assessment techniques using NMR, HPLC, mass spectrophotometry and optical detection have been developed specifically for islets and provide a unique set of investigational tools.

SERVICES

The ICFAC provides and assesses isolated islets from the following species:

1. rat
2. mouse
3. monkey
4. human

Rodent Islets:

The ICFAC has experience with Fischer, Sprague-Dawley, and Wistar rat islets, and C57 Black mouse islets. Other strains have also been successfully used. Investigators provide animals for rodent islet isolations.

Monkey Islets:

Primate pancreata are obtained by the ICFAC for islet isolation and affiliate use. *Macaca nemestrina* (pigtail monkey) pancreata are available an average of 1-2 times monthly for islet isolations from the Northwest Primate Research Center. These islets have been extensively characterized (Hagopian, et al Endocrinology 132:2674-81, 1993) and have similarities with human islets not shared by rodent islets.

Human Islets:

Human islets are prepared at the Islet Cell and Processing Laboratory at the Northwest Tissue Center/Puget Sound Blood Center. Availability will be dependent on clinical human islet transplantation. Supply is very irregular and cannot be predicted in advance. Users are notified of availability via email 12-24 hours prior to distribution.

Dispersed Islet Cells:

To facilitate experiments that require dispersed islet cells, islets are treated with collagenase and then mechanically disrupted. Cells are attached to glass slides allowing for binding and secretion studies and also patch clamping and ion pumping studies.

Flow Culture/Assessment of Islets.

A flow culture system has been developed to that allows continuous and non-invasive assessment of hormone secretion, metabolite release (lactate, pyruvate etc.), oxygen consumption, and cytochrome

redox state of islets or cell lines for up to 48 hours (1,2). In addition, it can temporally control the culture conditions maintaining the islets including chemical and gaseous composition of media, temperature and can accommodate non-recirculating, recirculating and co-culture modes.

Static Culture and Assessment of Islets.

As a complement to flow culture studies, culture and assessment of islets maintained statically are carried out in response to specific needs determined by the affiliate. More conditions can be handled statically so this mode is ideal for dose responses, particularly when cells need to be harvested for acquisition of sample. Examples are uptake and efflux of radioactively labeled compounds, effects of oxygen tension on cell viability and metabolic rates, production of low levels of metabolites or signaling molecules in the media, phenotyping functional characteristics of islets from genetically altered mice.

Single Islet Functional Imaging.

Functional imaging of single islets is carried out using a NIKON TE200 inverted fluorescence microscope. Autofluorescence of NADH, as well as fluorescence detection of islets stained with acridine orange/propidium iodide (membrane integrity), Rhodamine 123 (mitochondrial membrane potential), FURA (calcium flux), Annexin V (apoptosis) and other stains are offered.

General Information

Affiliates should phone the Associate Director to discuss their islet needs and future plans, especially noting number of islets needed, frequency needed, desired purity, desired species, post-isolation culture conditions, and methods of assessment.

Ian Sweet, Ph.D.

Core Director
(206) 685-4775
FAX 543-3169
isweet@u.washington.edu
Campus mailstop: 357710

Åke Lernmark, Ph.D.

Associate Core Director
(206) 543-5316
FAX 543-3169
ake@u.washington.edu
Campus mailstop: 357710

Department of Medicine (Box 356426)
University of Washington, Seattle, WA 98195

MOLECULAR GENETICS CORE (MGC)

The Molecular Genetics Core (MGC) offers several services to Affiliate Investigators:

- Consultation and training of staff and fellows in molecular techniques
- Technical assistance with experiments related to cloning, sequencing or gene expression as well as access to equipment such as thermocyclers
- Preparation of DNA, total RNA and mRNA as well as oligonucleotides for your molecular studies
- Gene bank analysis support and analysis of DNA and protein sequences
- Gene mapping and genomic scanning of human and rat genomes with microsatellite markers

The services are provided at the Health Science Center (K-165).

Åke Lernmark, Ph.D.

Core Director

R.H. Williams Professor of Medicine
R.H. Williams Laboratory, K-165
University of Washington, Box 357710
Seattle, WA 98195-7710
Phone: (206) 543-5316
Fax: (206) 543-3169
email: ake@u.washington.edu

Libby Rutledge, Ph.D.

Core Manager

Phone: 206-221-6508
email: erutledg@u.washington.edu