

**INTEGRATED ISLET DISTRIBUTION PROGRAM**  
**APPLICATION FOR IIDP SERVICES: HUMAN ISLETS FOR RESEARCH**  
**ISLET HANDLING TIPS**

**Background**

The purpose of this document is to provide investigators conducting IIDP approved research with a better understanding of how human pancreatic islets are handled, transported, and stored. These tips have been assembled by the IIDP and should be read by those who may have limited experience in the handling of human islets. The suggestions listed here have been provided by investigators<sup>1</sup> with numerous years of islet isolation and transplantation experience and is also based, in part, upon published literature (see below).

**General Information**

*Islet Requests:* The IIDP distribution centers will try to accommodate the number of islets requested by investigators and make every effort to distribute accurate islet equivalents (IEQs). It should be noted, however, that islet counting and aliquotting are difficult techniques to master. Because islets are divided into individual shipping containers, the number you receive may not be exactly what you need. Because of the fragility of the islets, there may also be some loss and break-up of islets during shipping. The loss of viability or reduction in islet integrity results from many factors including donor variables, isolation success, transportation time, and handling. It may be practical to ask for more islets than you anticipate needing for your study. After a few shipments, if you find that your original requests are inadequate, contact the Coordinating Center to increase your IEQ request. However, keep in mind the uniqueness and limits of IIDP resources.

*Rat vs. Human Islets:* Many investigators start their research using rat islets. You will find that human islets have a wider range in size distribution compared to rodent islets and usually secrete less insulin, as evidenced by stimulation and transplant studies. If this is your first attempt at working with human islets, we recommend that you do some background reading before deciding on the number of islets that you request.

*Islet Counting:* Human islets are counted by using a graded eyepiece in the microscope and some kind of grid to ensure that all islets in the aliquot are counted only once. The counting technique, conducted by taking a representative aliquot, is difficult to master. Make sure that your islet prep is thoroughly mixed before taking a sample to count. Islets settle very quickly. Dithizone (i.e. Diphenylthiocarbazone) diluted in DMSO or ETOH is a standard stain for counting islets. This stain will not allow you to assess the islet viability; rather it enables you to distinguish zinc containing beta cells from acinar tissue.

*Universal Precautions:* Upon arrival at your lab, the islets should be unpackaged and inspected to make sure that no containers were broken or have leaked during shipment. Universal Precautions should always be used whenever handling human islets. You are working with human tissue and although screening is done on all donors prior to isolation, care should always be exercised during handling.

*Breakage or leakage:* Universal Precautions should be taken in disposing of packing material if breakage occurs. The Coordinating Center should be contacted immediately to report any problems. Islets remaining in broken or leaking containers should be considered contaminated and results of further testing may be impaired.

**Use of Cultured Islets**

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<sup>1</sup> Primary author: Barbara J. Olack. Contributing authors: Abdulkadir Omer, Brad Richer, Gordon Weir

*Culturing of human islets:* Human islets can be held in culture for at least four weeks although some loss and degradation<sup>2</sup> will occur. For long term culture, it is recommended to maintain the temperature at 22-26°C. This will lengthen holding time by slowing islet metabolism. If, however, islets are used for metabolic or transplant studies immediately following a 22-26°C culture, they may not function properly. Two days at 37°C should allow islets to resume normal metabolic function. If islets are going to be used for studies within a week of receipt, culturing at 37°C can be started at the time of unpacking.

Standard culture media for human islets tend to be CMRL (Connaught Medical Research Laboratories) 1066 or RPMI (Rosewell Park Medical Institute) 1640, supplemented with a 10% serum additive, although many IIDPs have tried and succeeded with specialized formulas. CMRL is less attractive for lymphocyte survival and more desirable for transplant studies. Most clinical laboratories have switched to using Human Serum Albumin as a protein source for human islets, although Fetal Bovine Serum is also used in some metabolic studies. All IIDPs are currently using a CMRL base media for their islet shipments.

Most laboratories supplement their culture media with 2mM L-Glutamine. Antibiotics are generally not necessary if islets are handled properly, although the addition of Penicillin (100u/ml) and Streptomycin sulfate (100µg/ml) have been successfully used. There are many other additives in the literature that have been shown to prolong viability (vitamin E, selenium, Nicotinamide, etc); however, the results are laboratory dependent.

Islets should be transferred to vented T-flasks, culture bags or culture dishes at time of receipt and should be exposed to 95% air and 5% CO<sub>2</sub> in a standard incubator. Optimum density of islets in culture should also be considered. Early work by Lacy suggested to seed the islets in a density of 20 IEQ/cm<sup>2</sup> of surface area, which is still used by some during the culture of islets for basic research. However, in clinical practice, densities can range from approximately 250-400 IEQ /cm<sup>2</sup>. Culture medium can be changed 2-3 times per week.

*Transferring of islets:* Islets are groups of cells that must be handled with care to ensure their viability and integrity. It is recommended that a wide bore pipette is used for any transfer procedures. Pasteur pipettes, small bore tips used with automatic pipettors, and vigorous manipulation will break up the islet. An average islet, or one Islet Equivalent (IEQ), is 150µm in diameter but many will be smaller and some can be greater than 500 µm in diameter. Therefore, care should be taken in finding the right piece of equipment for handling. Gentle manipulation is always recommended.

*Storing of islets:* Islets cannot be frozen and thawed like other cell lines. Because of the larger mass of an islet, a slow, controlled cryopreservation technique must be used to ensure viability after thawing. Islets will hold in the above mentioned culture techniques for several weeks, so unless you have the equipment and an effective method for freezing islets, we recommend holding the islets in culture until your studies have been completed.

### **Use of Flash Frozen Islets**

Your shipment of flash frozen islets will be delivered in a Styrofoam box filled with dry ice. If there is no dry ice left in the shipping box, please contact the Coordinating Center and the islet shipping center immediately. The integrity of the islets should not be trusted and should be disposed of using Universal Precautions.

As soon as your frozen shipment arrives, the flash frozen islets should be stored at -135°C until your experiments begin. Islets can be thawed quickly in a 37° water bath where you will be able to retrieve islet cell fragments. THERE WILL BE NO VIABLE ISLETS IN A FLASH FROZEN SAMPLE. No cryoprotectants are used in the flash frozen process. If it is necessary for islets to be rinsed of media (e.g., to remove protein) before snap freezing, you must provide the Coordinating Center with a detailed protocol needed to conduct your studies.

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<sup>2</sup> Note: Formal degradation studies have NOT been performed on the human islets shipped through the IIDP program.

## References

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