

**ALBERTA DIABETES INSTITUTE
ISLETCORE WELCOME BOOKLET:
A USER GUIDE TO HUMAN ISLETS**



ALBERTA DIABETES INSTITUTE ISLETCORE WELCOME BOOKLET: A USER GUIDE TO HUMAN ISLETS

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INTRODUCTION

Welcome to the [Alberta Diabetes Institute IsletCore](#)! This booklet is intended to familiarize research groups with our program and the processes in place at our facility. We hope that you will also find it to be a helpful guide to handling human islets and obtaining the best results possible from this tissue.

About ADI IsletCore

[Alberta Diabetes Institute IsletCore](#) was launched in 2010 with the goal of isolating, distributing, and biobanking insulin-producing pancreatic islets from donor organs that cannot be used for clinical transplantation⁽¹⁾. Human islets are a valuable resource for the study of [islet cell biology](#) in health and diabetes and are in increasingly high demand. ADI IsletCore is located at the University of Alberta in Edmonton, Alberta, Canada and is one of the world's largest centres for islet isolation and distribution [exclusively for research](#). We currently provide services to over 160 research groups globally.

Our program has grown to include the provision of [biobanked samples](#) and additional pancreas-associated tissues. We are committed to accessible and transparent collection and reporting of human islet donor characteristics, and share data relating to basic donor information, quality measures, our biobank inventory, and functional analyses that are performed in-house.

We are proud to contribute to the important scientific discoveries of laboratories across the world and strive to support research and discovery in human islet biology well into the future. To learn more about acknowledging ADI IsletCore in your research publications, please see [Acknowledging Us](#) (page 15).



TEAM MEMBERS



Dr. Patrick MacDonald • Director

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General operations and collaborations.



Tina Dafoe • Program Coordinator

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Customer service and financial and operational management.



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Islet isolation, quantification, culture, and biobanking.



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Islet isolation, quantification, culture, and biobanking.



Aliya Spigelman • Technician

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Phenotyping, hormone secretion, and custom sample preparation.



Austin Bautista • Technician

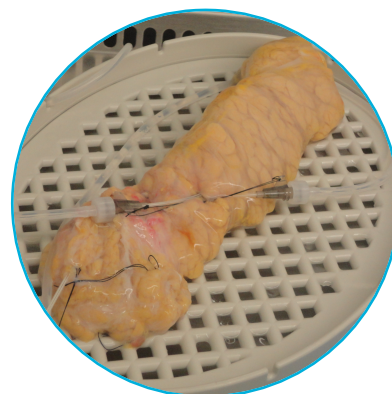
✉: austin2@ualberta.ca

Phenotyping, electrophysiology, and fresh pancreas slice preparation.

HUMAN ISLET ISOLATION

Isolation Protocol

The development of techniques to [isolate human islets from the pancreas](#) has been closely associated with the desire to use this tissue for transplantation in patients with type 1 diabetes. In 2000, the “[Edmonton Protocol](#)” for the isolation and transplantation of human islets was published by Dr. AM James Shapiro *et al.* in the New England Journal of Medicine⁽²⁾ and has subsequently been adopted as the standard protocol by leading islet transplantation centres worldwide^(3,4).



The process is adapted from that of Ricordi *et al.*⁽⁵⁾ and involves both mechanical and enzymatic digestion of the pancreas. The organ is perfused with collagenase and neutral protease enzymes via the ductal system, the tissue is agitated using a Ricordi chamber and the digested tissue is separated via a stainless steel mesh. Subsequent purification of the digested tissue is performed via continuous gradient centrifugation and fraction collection.

ADI IsletCore is fortunate to be closely associated with [Edmonton's Clinical Islet Laboratory](#) and the establishment of our facility has benefited greatly from the expertise of Drs. Shapiro, Kin, and the Clinical Islet Laboratory staff. Full details of our human islet isolation protocol can be found [here](#).

Organ Donors

ADI IsletCore processes pancreases obtained from cadaveric human organ donors across Canada. We are greatly indebted to these donors and their families, as well as to the [Organ Procurement Organizations](#) who coordinate the donation process. Consent for research use of the pancreas and associated tissues is obtained, and all donors undergo serology testing for infectious agents prior to donation. There are three types of pancreas donation:

- 1 Death Determination by Neurologic Criteria (DNC)
- 2 Death Determination by Circulatory Criteria (DCC)
- 3 Medical Assistance in Dying (MAiD)

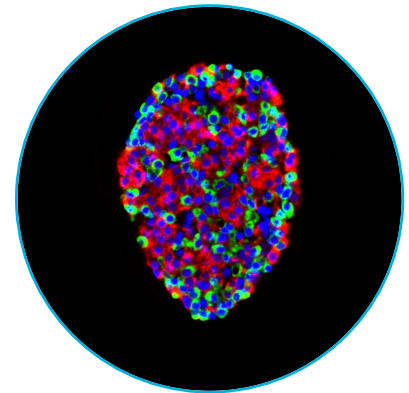
Islet isolation success is dependent on the [quality of the donor pancreas](#)⁽⁶⁾. Factors such as age, body mass index, medical history and type of donation all have an impact on islet yield and function^(1, 7-12). Extended cold ischemia time (CIT) also has an adverse influence on islets isolation⁽¹⁾: ADI IsletCore only accepts donors with a cold ischemia time of less than 24 hours and has a program-wide mean CIT duration of 14.4 +/- 0.3 hours.



COMPARING HUMAN & RODENT ISLETS

Morphology & Composition

Human and rodent islets are generally considered to differ in their [morphology](#)^(13,14), though there is some controversy surrounding these differences⁽¹⁵⁾. Briefly, mouse islets have a higher proportion of beta cells, compared to the proportion of beta cells in human islets.



Additionally, the beta cells in mouse islets appear to be [clustered](#) in the center of the islet, while the alpha and delta cells are found on the periphery. In contrast, alpha, beta, and delta cells are [dispersed evenly](#) throughout the human islet^(13,14), as seen in the picture above.

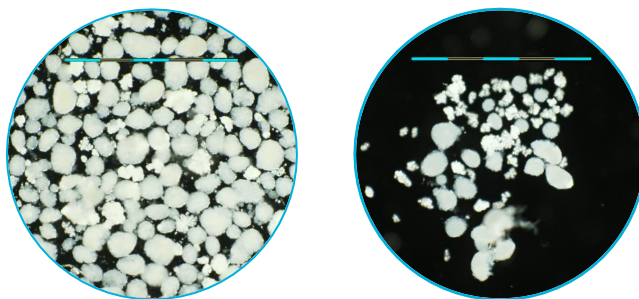
Size & Colour

From a practical perspective, one of the most noticeable differences when viewing human islets, compared to rodent islets, is the [significant size variation](#) both within preparations and between donors. When comparing islets from multiple human donors, the islet particle index (IPI, described below) can be used to help determine the expected average islet size.

Another characteristic that may be apparent when viewing human islets is the [colour](#). Mouse islets tend to appear bright “white” and have a good contrast between the islets and the acinar. In contrast, islets from some human donors may appear somewhat grey, less opaque or with poorer contrast between the acinar and the islets. Additionally, human islets are often less spherical, and may exhibit a more irregular border than mouse islets.

Other Differences

Human islets tend to be [more fragile](#) and may become fragmented if not handled gently. It is also important to note that mouse islets are typically cultured in 11.1 mM glucose media in contrast to human islets, which are cultured in 5.5 mM glucose media.



Hand-picked human (left) and mouse (right) islets. Scale bar = 2 mm.



HUMAN ISLET QUANTIFICATION

Actual Islets Versus Islet Equivalents (IEQ)

Human islets exhibit a broad size distribution, with each islet containing various quantities of cells. Thus, the actual number of islets present is not necessarily representative of the number of cells present. The volumetric adjustment of human islets of different sizes to standardized [Islet Equivalents](#) (IEQ) facilitates consistency when assessing islet isolations from different donors and centres⁽¹⁶⁾.

One IEQ is the [standard unit of human islet volume](#) and refers to a conceptually idealized islet with a diameter of 150 μm , as set at the Second Congress of the International Pancreas and Islet Transplantation Association. An islet with a measured diameter of less than 150 μm is less than one IEQ, while one with a diameter of greater than 150 μm represents greater than one IEQ. The method for calculating IEQ is described in [Working with Human Islets – Counting](#) (page 10).

If you are more familiar with working with ‘actual’ islets (i.e. hand-picked islets, as is common with rodent islets) and require a certain number of islets for your experiment, we suggest [multiplying this number by 10](#) to determine the number of IEQ to request.

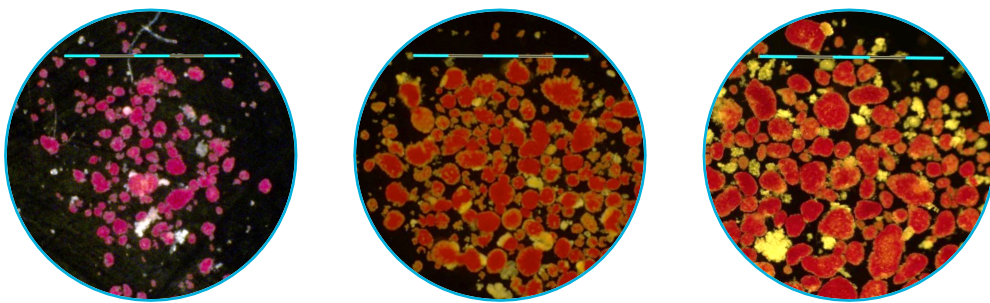
For example, if you need 500 hand-picked islets for your study, a good starting point is to request 5,000 IEQ. This should be sufficient for you to hand-pick the required number of islets, although this multiplication factor may vary slightly based on islet size (see below).

Islet Particle Index (IPI)

An associated measure is [Islet Particle Index](#) (IPI, also known as Islet Size Index or Islet Index). This is defined as the number of IEQ divided by the number of [islet particles](#) (IP), and gives an indication of the size of the islets relative to a standard 150 µm diameter IEQ.

$$IPI = \frac{IEQ}{IP}$$

An IPI value of less than 1.0 means the islets tend towards the smaller end of the size distribution spectrum, whereas those with an IPI of greater than 1.0 tend towards the larger size range. The size of an islet has implications for diffusion of oxygen and nutrients *in vitro*: the larger the islet index, the more susceptible the prep is to islet loss in culture⁽¹⁷⁾. This attrition is also reflected in a decrease in IPI following islet culture, as the larger islets are the ones that are primarily lost.



Different islet particle indices. From left to right: 0.4, 1.0, 2.6. Scale bar = 2 mm.

For any given quantity of IEQ, a high IPI value typically indicates fewer “actual” islets can be hand-picked, although each islet may be larger than usual. Thus, the IPI should be taken into consideration when determining how many IEQ to request for your experiment.

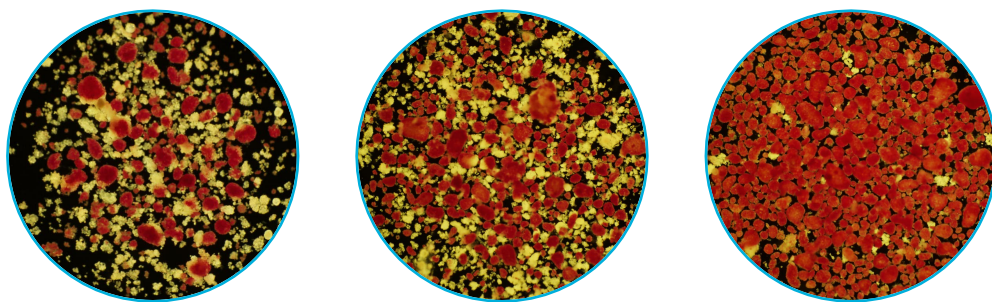
FROM OUR BENCH TO YOURS

Requesting Islets

A [notification email](#) is sent to all recipient groups when a preparation of human islets is available. This email contains a link to the [online request form](#), estimated shipping dates, and the following donor and preparation information:

- Donor ID
- Donor age
- Donor sex
- Donor body mass index
- Donor HbA1c measurement
- Donor HLA-A2 status
- Donation type
- Percent purity
- Percent trapped
- Islet particle index
- Pre-shipment culture time
- Dithizone-stained images of the isolated islets

Through this process, each group can assess the suitability of the preparation prior to placing a request. We do our best to accommodate all requests, taking into account any previously unfulfilled requests, the order in which requests are received, and any time-sensitive needs.



Purity estimates are based on the volume of islet versus acinar tissue. From left to right: 50%, 75%, 95%.

Islets are [requantified](#) on the morning of distribution and every effort is made to distribute accurate islet equivalents. It should be noted, however, that islet counting and aliquoting are challenging techniques with high levels of [user-dependent subjectivity](#).

Many factors impact the number of islets you may receive, including loss and fragmentation of islets during shipping. Decreased viability or reduction in islet integrity can also result from factors including donor variables, pancreas processing, shipping duration, and handling. To mitigate these factors, it may be pragmatic to start by asking for more islets than you anticipate needing. After a few shipments, you will likely have a better idea whether your needs are being met and can adjust your requests accordingly.

Shipping Islets

Prior to distribution, islets are [cultured](#) in our facility for approximately 12 to 96 hours, depending on the timing of the isolation (see [Working with Human Islets - Culturing](#), page 9). On the day of distribution, IEQs are re-quantified and samples are aliquoted into 50 mL tubes, with a maximum of 40,000 IEQ per tube. The samples are then brought to volume with [Supplemented CMRL](#) (Connaught Medical Research Laboratories) media and packaged for shipment at ambient temperature. The islets are considered “exempt human specimens” and are packaged in accordance with IATA Dangerous Goods Regulations. Tubes are packaged on their side to prevent islet pelleting and a temperature indicator is included with the shipment.



We ship our islets via [FedEx overnight service](#) within Canada and the USA. Shipments overseas typically arrive within [48 hours](#). Shipping delays do occasionally occur and are unfortunately beyond our control. The tracking information is provided to the recipient. We recommend following this closely and contacting FedEx immediately if any delay is noted.

Receiving Islets

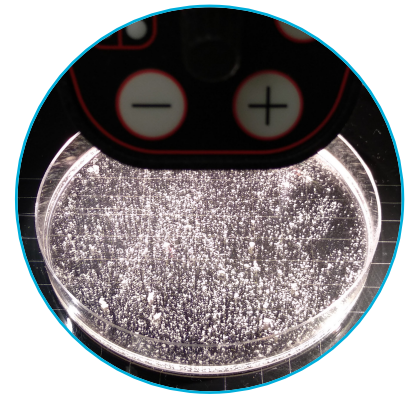
Although ADI IsletCore's donors are [serology tested](#) prior to islet isolation, universal precautions and best practices should be used when handling any human tissue. The use of [aseptic technique](#) is strongly recommended to prevent microbial contamination of the islets.

Upon receipt of your shipment, unpackage the tube immediately, taking note of the temperature monitor. The tube should be checked for damage or leakage and the sample visually inspected for microbial contamination. Record the Donor ID number and proceed to process or culture the islets right away. Once you have had the opportunity to assess the quality of your islets, we appreciate you [providing feedback](#) via our [online feedback form](#) to assist us in improving our service.

WORKING WITH HUMAN ISLETS

Handling

Upon receipt, the islets may be used immediately or placed into culture (see [Working with Human Islets – Culturing](#), below). In either case, they should be retrieved from the shipping tube by centrifugation at 200 x g for three minutes. The supernatant can then be removed, and the tissue resuspended in an appropriate volume of fresh media and handled using a serological or wide bore pipette.



When transferred to a non-treated culture plate or flask, the islets can be [visualized](#), without staining, under a stereoscope at 100x to 400x magnification. To improve contrast between islets and acinar tissue, we recommend placing the dish on a dark background and applying light from the sides.

Individual islets can then be [“picked”](#) using a pipette and transferred to other plates, petri dishes, or tubes, as appropriate. A P10 pipette is typically used for this task, but very large islets may necessitate the use of a larger bore pipette. Gently swirling the dish helps concentrate the islets towards the centre, moving the acinar tissue towards the edges, which can increase the speed at which islets are located and selected. Occasional rinsing, remixing, and reswirling is helpful in separating islets from acinar tissue.

Culturing

Prior to shipping, human islets are held in culture at 22°C, 5% CO₂, to [slow metabolism and reduce islet loss](#). [Supplemented CMRL](#) media is used and the islets are plated in 150 mm non-treated culture dishes at a maximum density of 250 IEQ/cm². This is also our shipping media of choice.

Upon receipt of the human islets at your facility, you may wish to place them in culture to accommodate your experimental timeline. Optional culture media include [CMRL](#), [RPMI 1640](#) (Roswell Park Memorial Institute), or [DMEM](#) (Dulbecco's Modified Eagle's Medium) containing 5.5 mM glucose and supplemented with serum (human or bovine complete serum or serum albumin), glutamine, antibiotics, and other additives as desired.

Prior to functional studies, we recommend culturing the islets at 37°C for at least 18 hours to allow [normal metabolism](#) to resume. Hand-picking islets to purity prior to culture at 37°C is recommended to minimize the release of cytokines from exocrine components. Our standard media for 37°C culture prior to functional assay is DMEM containing 5.5 mM glucose, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

[Non-treated culture dishes](#) are recommended to minimize adhesion of islets to the surface of dish. The islets should be cultured in a 5% CO₂ environment at a maximum density of 250 IEQ, or 25 hand-picked islets, per square centimeter. Media changes can be performed every three to four days. The rate of decline of islet quantity and quality in culture depends on many factors, but under these conditions, we have experience successfully maintaining islets in culture for [up to seven days](#).

Sampling

In order to take a representative sample for assessment, it is important to ensure that islets of diverse sizes are [resuspended](#) throughout the aliquot by mixing the aliquot thoroughly. This can be achieved by inverting the aliquot in its 50 mL conical tube three to four times, or by pipetting with a serological or wide-bore pipette prior to sampling.

Samples should be taken [immediately following resuspension](#), before any resettling occurs. A wide-bore pipette tip should be used to prevent applying shearing forces to the islets, and the sample should be taken from the middle of the aliquot.

Counting

Human islets are counted by using a [microscope with an eyepiece reticle](#) for islet sizing, in conjunction with a grid placed under the sample to ensure that each islet is only counted once. The islet sample is first stained with the zinc-binding dye, [dithizone](#) (DTZ), which enables [visual identification](#) of islets by their red colour. Acinar tissue, which lacks zinc-containing beta cells, remains unstained.



For [islet quantification](#), ADI IsletCore assesses a sample representing 0.05% of the total preparation (50 µL of 100 mL). To quantify islets in your own lab, this proportion should be adjusted based on the number of IEQ expected. It is important to count 50-100 islet particles (IPs) to ensure that a representative size distribution is achieved.

Be careful to [mix the preparation well](#) without swirling before taking the sample for counting. Briefly incubate the sample with DTZ and assess the number of islets per size bracket, using the eyepiece reticle. It is important to move the dish very carefully during counting to prevent the islets from shifting position. The complete protocol—with associated counting sheets and formula—is available [here](#). A [nine-channel benchtop cell counter](#) can be used to facilitate counting, as pictured below.



A nine-channel benchtop cell counter is used to facilitate counting.

The same sample may be used for assessment of additional factors such as purity, acinar trapping, and islet morphology, as described in the [protocol above](#). Additional samples may be taken for other quality assessment purposes, as suggested [here](#).



TROUBLESHOOTING

Common Concerns

The most common concern we hear from recipient labs is related to the [number of islet equivalents received](#). As noted in [Human Islet Quantification](#) (page 5), quantifying IEQ is highly challenging, with high levels of user-variability, and many additional factors may impact the survival of islets during transit. It may be pragmatic to ask for more islets than you anticipate needing, to account for these factors. We always strive to provide [10-15% additional IEQ free of charge](#) to account for potential losses, when the preparation yield allows.

Occasionally, recipient labs report that the [cold-mark temperature monitor](#) has been triggered. While this indicates that the monitor has been exposed to temperatures lower than 2°C, our experience suggests that the larger thermal mass of the 50 mL islet aliquot is less likely to have dropped to this temperature. In most cases, the islets appear in good condition and it is appropriate to proceed with experiments.



A temperature monitor showing the cold (left) and warm (right) temperature triggers.

Some islet preparations have a tendency to “[chain](#)” or adhere together in clumps. Often, these can be disrupted by gentle pipetting with a wide-bore pipette tip (e.g. P1000). Different media may affect the level of human islet adhesion, with [formulations reported to reduce chaining](#).

While aseptic technique is employed during all handling of islets in the ADI IsletCore facility, occasionally islet preparations exhibit microbial contamination. If you suspect that your islet shipment is contaminated, please contact [Tina Dafoe](#) immediately so that other islet recipients can be notified.

Feedback and Credit Policy

As we strive to ensure that the islets we provide are of the [highest quality](#), we appreciate your feedback on each preparation that you receive. This can be provided quickly and conveniently using our [online feedback form](#). You will receive an email prompting you to complete this following each shipment you receive. If your shipment is in any way unacceptable and you are seeking a refund, you will be asked to send a digital photograph of the islet preparation to substantiate your claim.

We encourage our recipients to carefully consider whether the quality of the sample is truly degraded before requesting a credit. Please find our full credit policy [here](#).

OUR TISSUE BIOBANK

We maintain a biobank of human pancreas and islet samples including:

- Formalin-Fixed Paraffin-Embedded (FFPE) sections
- Cryopreserved islets
- Snap-frozen islets

Samples from other tissues, including spleen, adipose, intestine, lymph nodes, and blood can also be collected upon request. If you are interested in custom processing (e.g. optimal cutting temperature [OCT]-compound embedding, electron microscopy [EM] fixation), please [contact us](#).

Cryopreserved Islets

Access to human research islets remains a limiting factor in many scientific studies⁽¹⁸⁾ and the inherent difficulties of distribution and culture time only add further to the value of any human islet tissue obtained. [Biobanking](#) has the potential to overcome many of these issues and enables the researcher to simultaneously obtain multiple samples from donors with the desired criteria for their experiments. In many cases, the [successful retention of physiological function](#) in frozen-thawed islets is desirable.

ADI IsletCore maintains a biobank of cryopreserved islets from which some functional viability has been demonstrated⁽¹⁹⁾. The [cryopreservation process](#) involves rate-controlled cooling, using dimethyl sulfoxide (DMSO) as a cryoprotectant to prevent any cellular damage. We upkeep records of our cryopreserved samples, as well as the number of IEQs per vial.



While we have demonstrated electrophysiological, insulin secretory, and transplantation potential of these samples, cryopreservation has some impacts on normal function. In addition, handling cryopreserved islets can be challenging. As such, the tissue is best suited to the study of [cellular extracts](#), although other procedures (e.g. islet dispersion and fluorescence-activated cell sorting [FACS]) have been successfully performed by groups using our samples.

Thawing Cryopreserved Islets

Cryopreserved islets are shipped on dry ice and may be stored in liquid nitrogen until use. To retrieve viable and functional islets, the ADI IsletCore [thawing protocol](#) must be followed. This procedure removes the [cryoprotectant](#) by serial dilution, which prevent osmotic shock and maintains the membrane integrity of the islet cells.

Once thawed, islets should be [hand-picked to purity](#) to prevent chaining or clumping, to which these preparations are particularly susceptible. Cryopreserved islets are particularly fragile and do not tolerate culture well, so we recommend using them as soon as possible after thawing.

Snap-Frozen Islets

ADI IsletCore maintains a biobank of snap-frozen samples that are ideal for the study of islet cell extracts. Each vial contains a pellet of [2,000 IEQ](#). Samples are pelleted via centrifugation at 200 x g for one minute, the media removed via aspiration, and the pelleted tissue is plunged into liquid nitrogen, before being stored at -80°C. No cryoprotectants are used in the snap-freezing process and the samples do not contain viable islets.

Snap-frozen islets are shipped on dry ice and may be stored at -80°C until ready for use. Vials should then be rapidly thawed at 37°C, and islet fragments and extracts can then be retrieved.

WHAT WE OFFER

at ADI IsletCore



FRESH ISLETS

High purity, research-grade human islets from donors with and without diabetes at cost-recovery pricing.



FROZEN ISLETS

Cryopreserved and snap-frozen human islets for expression, -omics, and select functional studies.



BLOCKS & SLIDES

Paraffin-embedded pancreas biopsies and isolated islets. Custom fixation available upon request.



TISSUE SAMPLES

Samples of intestine, spleen, adipose, lymph nodes, and blood. Custom processing available upon request.



FUNCTIONAL DATA

Whole islet insulin secretion and single beta-cell function assays performed on every preparation.

ACKNOWLEDGING US

Proper acknowledgement of ADI IsletCore is important in order for us to continue to justify grant applications and requests for subsidies to support the program and keep user fees as low as possible. It is also important to acknowledge the organ procurement organizations for their tireless efforts in obtaining organs for our program and your research.

We appreciate you using the following wording to acknowledge the use of materials provided by ADI IsletCore:

Human islets for research were provided by the Alberta Diabetes Institute IsletCore at the University of Alberta in Edmonton (<http://www.bcell.org/adi-isletcore.html>) with the assistance of the Human Organ Procurement and Exchange (HOPE) program, Trillium Gift of Life Network (TGLN), and other Canadian organ procurement organizations. Islet isolation was approved by the Human Research Ethics Board at the University of Alberta (Pro00013094). All donors' families gave informed consent for the use of pancreatic tissue in research.

If you'd like to cite the resource, we suggest:

Lyon J *et al.* (2016) Research-focused isolation of human islets from donors with and without diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology*, 157(2): 560-569.

ACKNOWLEDGEMENTS & REFERENCES

Acknowledgements

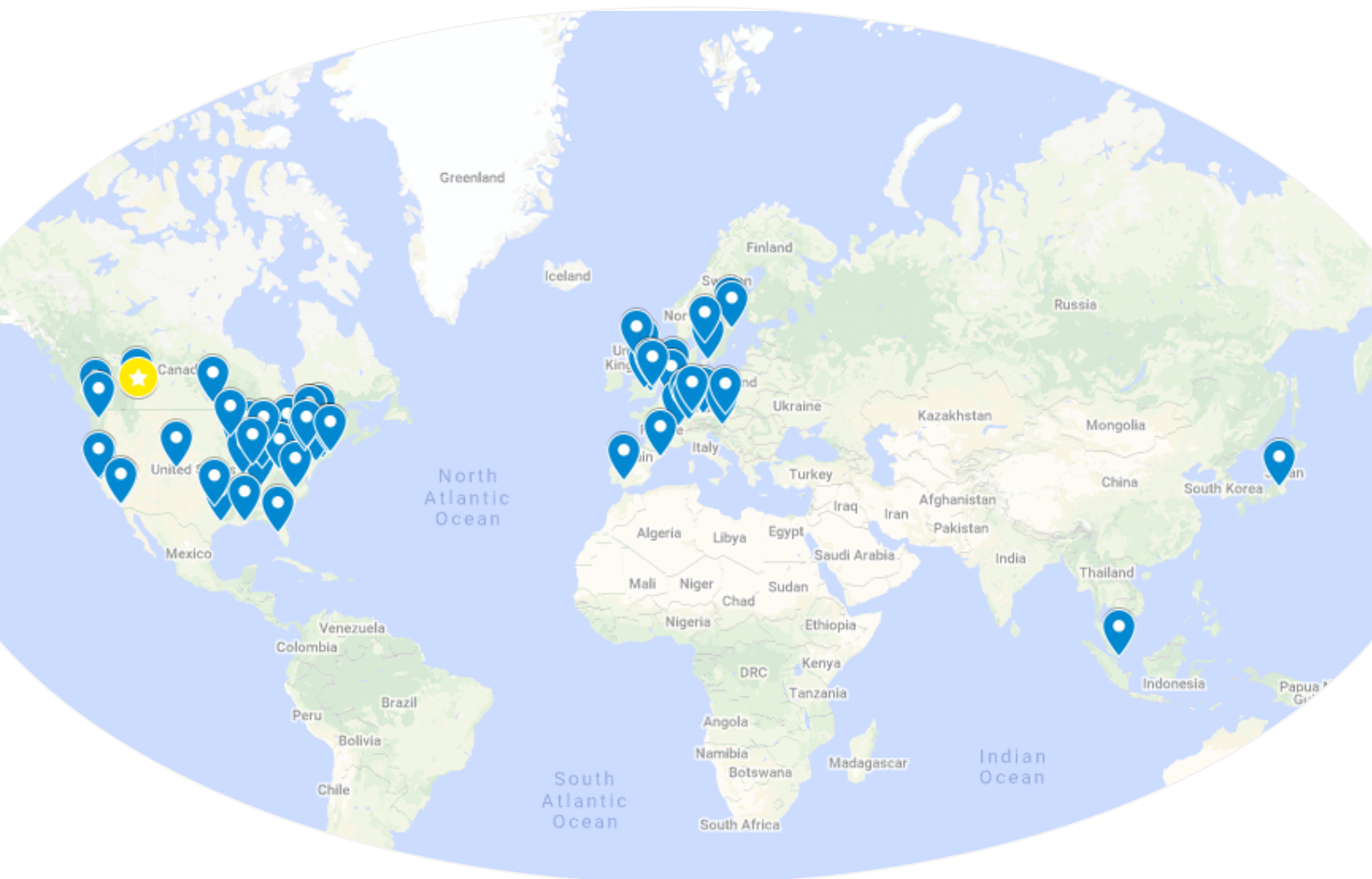
The ADI IsletCore team are indebted to Dr. Tatsuya Kin, Dr. AM James Shapiro, and the staff at the [Clinical Islet Laboratory in Edmonton](#), who are continually generous with their expertise in human islet isolation. We thank the [Integrated Islet Distribution Program](#) (IIDP) for inspiration in the creation of this Welcome Booklet, in particular Barbara Olack for her review of this document.

We are grateful for the assistance of the [Human Organ Procurement and Exchange](#) (HOPE) program, [Trillium Gift of Life Network](#) (TGLN), and other [Canadian Organ Procurement Organizations](#) in coordinating the donation process. Most importantly, we would like to thank the [organ donors and their families](#) for their priceless gift in support of diabetes and islet biology research.

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