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Standardized Transportation of Human Islets: An Islet Cell Resource Center Study of More Than 2,000 Shipments

John S. Kaddis, Ph.D.[1], **Matthew S. Hanson, Ph.D.**[2], **James Cravens, M.P.H.**[1], **Dajun Qian, Ph.D.**[1], **Barbara Olack**[1], **Martha Antler**[1], **Klearchos K. Papas, Ph.D.**[3], **Itzia Iglesias, M.S.**[4], **Barbara Barbaro, Ph.D.**[5], **Luis Fernandez, M.D.**[2], **Alvin C. Powers, M.D.**[6,7], and **Joyce C. Niland, Ph.D.**[1,*]

[1]Department of Information Sciences, City of Hope, Duarte, CA, USA

[2]Department of Surgery, University of Wisconsin, Madison, WI, USA

[3]Department of Surgery, University of Minnesota, Minneapolis, MN, USA

[4]Department of Diabetes and Metabolic Diseases Research, City of Hope, Duarte, CA, USA

[5]Division of Transplant Surgery, University of Illinois at Chicago, USA

[6]Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[7]VA Tennessee Valley Healthcare System, Nashville, Tennessee, USA

Abstract

Preservation of cell quality during shipment of human pancreatic islets for use in laboratory research is a crucial, but neglected, topic. Mammalian cells, including islets, have been shown to be adversely affected by temperature changes in vitro and in vivo, yet protocols that control for thermal fluctuations during cell transport are lacking. To evaluate an optimal method of shipping human islets, an initial assessment of transportation conditions was conducted using standardized materials and operating procedures in 48 shipments sent to a central location by 8 pancreas-processing laboratories using a single commercial airline transporter. Optimization of preliminary conditions was conducted, and human islet quality was then evaluated in 2,338 shipments pre- and post-implementation of a finalized transportation container and standard operating procedures. The initial assessment revealed that the outside temperature ranged from a mean of −4.6±10.3°C to 20.9±4.8°C. Within-container temperature drops to or below 15°C occurred in 16 shipments (36%), while the temperature was found to be stabilized between 15–29°C in 29 shipments (64%). Implementation of an optimized transportation container and operating procedure reduced the number of within-container temperature drops (≤15°C) to 13% (n=37 of 289 winter shipments), improved the number desirably maintained between 15–29°C to 86% (n=250), but also increased the number reaching or exceeding 29°C to 1% (n=2; overall $p<0.0001$). Additionally, post-receipt quality ratings of excellent to good improved pre- vs. post- implementation of the standardized protocol, adjusting for pre-shipment purity/viability levels ($p<0.0001$). Our results show that extreme temperature fluctuations during transport of human islets, occurring when using a commercial airline transporter for long distance shipping, can be controlled using standardized containers, materials, and operating procedures. This cost-effective and pragmatic standardized protocol for the transportation of human islets can potentially be adapted for use with other mammalian cell systems, and is available online at: http://iidp.coh.org/sops.aspx.

[*]**Corresponding Author:** Joyce C. Niland, PhD, Chair, Department of Information Sciences, City of Hope National Medical Center, 1500 East Duarte Road, Duarte CA 91010-3000, USA, Phone 626-256-4673 ext. 63032; Fax 626-301-8802, jniland@coh.org.

Keywords

human Islets; shipping protocol; temperature control; diabetes; insulin

INTRODUCTION

Human pancreatic islets play a vital role in laboratory research and are being used in islet transplantation for a subset of individuals with type 1 diabetes (22). Because human islet isolation is expensive (12) and requires specialized facilities staffed with experienced personnel, the need for islet shipping protocols to extend the services of established pancreas processing centers to distant clinical transplant centers and basic science laboratories is growing (10). As the frequency, duration, and distance of islet shipments increase (22,36,54), so too does the concern over adverse extreme temperature effects on preparation quality and function. Changes of at least ± 20°C during intra- and intercontinental shipments using a commercial airline transporter have been documented (19,43).

A number of published articles have reviewed the cold (1) and heat (40) shock responses of mammalian cells, including studies on gene expression changes during periods of thermal fluctuation (50,51). Such reports suggest a need to account for thermal effects on cell biology experiments, including any undesirable temperature changes during transportation of human islets. Unlike tumor-derived or artificially immortalized cell lines (16), primary human pancreatic islets have a finite lifespan and cannot be readily stored or cultured for long periods of time, although certain exceptions exist (26,44).

Exposure of islets to moderate hypothermia (22–24°C) remains the most widely used non-physiological temperature during cell culture, and has been shown to eliminate or reduce the number of passenger leukocytes (27) and intraislet lymphoid cells (28) present. The beta-cell damaging effects of interleukin-1-mediated inducible nitric oxide synthase expression by resident macrophages, during culture, have now been documented (2,49). While the benefits, and consequences of culture at 22–24°C vs. 37°C have been reviewed by Murdock and colleagues (34), little is known about the effects of prolonged extreme hypothermia on isolated human islets. In proliferating mammalian cells, culture at 4°C reduces cell growth and viability (17), and leads to morphological and nuclear alterations, including bleb formation, DNA fragmentation, and cell apoptosis (39). Cell cycle progression has been shown to be altered in a number of cell lines grown at <20°C (42). Conversely, a mild reduction of culture temperature, from 37°C to 32°C, prevented apoptosis by a variety of cellular stressors (45).

Hyperthermic conditions also have been shown to alter cellular survival and function. Brandhorst and colleagues exposed pig islets to mild hyperthermia (43°C) and found that although there was an increase in the resistance to inflammation by in vitro stimulation when compared to controls at 37°C, there also was an enhancement in the number of apoptotic proteins detected in vitro, as well as a reduction in the early survival of xenografts (5–7). In proliferating mammalian cells, mild hyperthermia also was shown to diminish the inflammatory response of cytokine-stimulated cell lines (13). Following transplantation, islet graft angiogenesis and revascularization was reduced in heat shock preconditioned hamster islets vs. controls (48). In heat-shocked human islets, in vitro protection against cytokine-induced damage and nitric oxide radicals has been reported (46). Although variation in response to hyperthermia may in part be explained by different levels of species-specific basal expression of heat shock and other cellular stress response proteins (55), as well as core body temperature differences across animals (21,31,52), the effects of human islet exposure to non-physiological temperature increases is not known.

Recent studies have employed the use of 22°C following a short initial culture period of 37°C (9,14,15). While the concept of reducing human islet culture temperature was introduced more than two decades ago (41,47), common, affordable, and optimal transportation protocols that target and maintain thermal stability have not been developed. Moreover, studies examining within-container temperatures during human islet shipment are lacking.

We therefore conducted a series of experiments to design, optimize, and implement a method for long-distance transport of human pancreatic islets using a commercial airline transporter and standard operating procedures (SOPs). The goal was to stabilize the temperature within the shipping container during long distance transport to minimize extreme fluctuations exceeding ± 7°C of the selected reference value at 22°C. This study was performed by the National Islet Cell Resource Center (ICR) Consortium, a multi-center initiative from 2001–2009 focused on improving human islet isolation and transplantation technologies. After conducting experiments to develop a standardized set of shipping materials and operating procedures, a total of 2,338 human pancreatic islet shipments, sent to investigators in North America, Israel, and Australia from 14 different laboratories across the U.S., were assessed pre- (n=906 shipments) versus post- (n=1,432) implementation of a uniform shipping SOP to evaluate stability of the temperature during shipping, and the quality of the preparations upon arrival.

MATERIALS AND METHODS

Preliminary Shipping Evaluation and Equipment Testing

For the preliminary testing of standardized processes and equipment, a total of 8 facilities from 7 States (see list in the acknowledgements) each shipped 6 containers to the coordinating center at the City of Hope in Duarte, CA. Islet containers were prepared following an initial SOP. Fed-Ex overnight service was used by each participating laboratory twice a week for three consecutive weeks during winter, from January 29, 2007 through February 16, 2007, and included at least one “hub-stop”, defined here as an en-route landing and subsequent departure at an airport, where cargo is exchanged, prior to reaching the destination location.

We generated and assessed temperature and transportation data collected during the shipment of each package. Each container included: a reusable temperature and pressure data logger, a disposable temperature indicator strip, 2 ambient temperature stabilization gel-packs, a 240mL cell culture bag, an absorbent cotton pad, and packing peanuts inside a polystyrene foam inner container with a corrugated cardboard outer box (Table 1). Ambient temperature stabilization gel-packs were prepared according to SEBRA manufacturer protocol for temperature protection of platelet concentrate units at 20–22°C (Haemoentics Corp.; Braintree, MA). Human islets were not used for the preliminary shipping evaluation experiments.

The reliability of temperature data loggers used for monitoring values inside the shipping container during transportation were evaluated prior to initiation of this study using sensitivity and precision statistics (Supplemental Table 1)[1]. Readings for these experiments were logged every 2 minutes for temperature and 8 minutes for pressure for the duration of each shipment.

The data logger demonstrated high sensitivity in both trial runs, as measured by the linear agreement between all pairs of temperature probes tested (r=0.97±0.01 in Run 1 and

[1]All supplemental materials are available at: http://iidp.coh.org/shipping_study.aspx

0.93±0.05 in Run 2). The maximum variation seen in temperature monitor readings was less than 1°C from the mean value, signifying moderate precision in all trial runs (0.49°C ± 0.32 in Run 1; 0.98°C ± 0.49 in Run 2). The coefficients of variation for the temperature monitors were 3.20% ± 2.22 in Run 1 and 3.74% ± 2.01 in Run 2, signifying negligible dispersion of temperature reading measurements within and between all trial runs.

Optimization of Temperature Control During Mock Islet Shipments

The use of an ambient temperature stabilization gel-pack inside an islet shipping container to control cold and hot weather changes has been previously reported (18). To optimize deployment of this product, temperature control inside a standardized islet shipping container was assessed using 0, 2, 4, 6, and 8 gel-packs under fixed and alternating external temperature environments. Fixed conditions included exposure to −20°C, 4°C, room temperature (16–25°C), and 37°C. Alternating conditions included temperature variations from −20°C to 37°C and vice versa. Temperature readings for these experiments were logged every 10 seconds for a minimum of 18 hours.

Validation Study to Assess the Standardized Shipping Container and SOP

From February 12, 2004 – December 31, 2010, 4,068 shipments of human islets were provided to investigators by the ICR Basic Science Human Islet Distribution Program, as previously described (22), or the Integrated Islet Distribution Program (IIDP). Laboratory experiments being conducted using the islets have been previously described in an analysis of research projects supported by the ICR program (22).

Of 4,068 islet shipments, 683 were excluded because they were either shipped using a pilot version of the standardized protocol in the pre-study period, or during the transition period after release of the SOP, but before complete compliance with the protocol. An additional 335 were excluded because they involved direct pick-ups that were not shipped using a commercial airline transporter. Finally, 712 were excluded due to missing or incomplete pre-and/or post-shipment islet assessment data. Therefore, information from 2,338 shipments was used for this validation analysis, including 906 prior to the standardized protocol being in place, and 1,432 which used this SOP to optimize temperature control.

Study Endpoints

The primary outcome variable was within-container transport temperature, measured using either a reusable temperature data logger (continuous variable) or disposable indicator (categorical data). Human islet quality rating was used as a secondary study endpoint to qualitatively measure the satisfaction of the receiving investigators with the cellular preparation for laboratory experimentation post-shipment. Researchers rated the shipped islets as excellent, good, fair, or poor, using the testing method(s) most appropriate to their experimental needs. Their ratings were based on a) assessment of count, purity, and/or viability, and/or b) usability of the preparation in laboratory experiments. For analysis purposes we collapsed the islet rating variable into two categories, “excellent to good” and “fair to poor” for a more global indication of the quality of the shipped islets. While it was desirable to impose a single uniform assessment method, given the large number of investigators receiving shipments (n=123), it was not feasible to standardize the post-shipment methods used by recipient laboratories to rate the islets. Moreover, we have previously shown that even when highly experienced individuals received the same exact product and were trained to utilized standardized islet assessment methods (in this case for islet counting), statistically significant variation in islet assessments was seen for up to 1/3 of all participating laboratories (25). Post-shipment ratings were stratified by pre-shipment preparation quality to control for differences in purity and viability of islets prior to distribution. We defined superior islets as those with both ≥85% purity and >90% viability

(n=700). Average islets were defined as those with <85% purity, ≤90% viability, or both (n=1638).

Statistical Methods

Percentages are reported for categorical variables. For continuous variables, the measure of central tendency was described using both the mean ± 1 standard deviation (SD) and median plus range (min, max). Variation of temperature during transportation was assessed by evaluating temperature changes during each shipment, and rate of change per minute. Assessment of agreement between reusable and disposable temperature monitors was evaluated using the kappa statistic (29). Due to small cell sizes, Fisher's exact test was used to examine differences in temperature probe activations across seasonal variations. The Cochran-Mantel-Haenszel test statistic was used to examine the post-shipment islet quality ratings before and after implementation of the standardized islet shipping protocol, controlling for pre-shipment purity/viability, after testing for homogeneity of quality ratings across strata via the Breslow-Day statistic.

Analysis of the association of shipping conditions with temperature drops (≤15°C) was performed using univariate logistic regression modeling, with corresponding odds ratios (ORs), and 95% confidence intervals (CIs) reported. Variables with a p-value <0.20 from the univariate logistic regression analysis were considered for inclusion in the multivariable model. Stepwise logistic regression was used to select the independent factors. Statistical significance was defined as a two-sided p value <0.05. All analyses were performed using SAS software version 9.1.3 12 (SAS Institute, Cary, NC).

RESULTS

Preliminary Shipping Evaluation

Eight ICR laboratories completed a total of 48 shipments for the preliminary evaluation of standardized shipping processes and equipment (Table 2). Complete data for all parameters of interest were obtained in 44 of 48 shipments (92%). Compliance to the shipping SOP was documented via: a) digital photography of the islet container during different stages of the package preparation process (images not shown), and b) monitoring of the gel-pack temperature during packaging of the container (Supplemental Table 2). Gel-packs were warmed to room temperature during packaging, (defined as >20.0 to 26.0°C) in 29 of 46 shipments (63%). Temperature of the gel-packs fell below 20.0°C during packaging of 17 shipments (37%).

Temperature fluctuations inside the container during shipping ranged from an overall low of 4.7°C to a high of 26.6°C, with a mean temperature change of 7.5°C ± 3.8 (range: 2.1–18.9, Table 3). Maximum rate of temperature change in the container was found to be 0.32°C/minute ± 0.24 (range: 0.05–1.2°C/minute). Ambient temperature at departure, arrival, and hub cities ranged from −19.4 to 31.7 °C. Forty-two of 46 shipments (91%) were delivered on time (≤28 hours), with a mean shipping distance of 1,851 miles ± 726 (range: 38–2,544 miles). Temperature profile line plots by ICR center and shipment are shown in Supplemental Figure 1.

The performance of disposable temperature indicators relative to reusable probes was evaluated (Supplemental Table 3). There was statistically significant agreement between disposable temperature indicators and reusable temperature probes (Kappa=0.50; 95% CI: 0.25 to 0.74; p<0.0001). As the disposable temperature indicators were 50 times less expensive than the reusable probes and did not require additional data extraction software/hardware, we therefore utilized the more economical and practical strips for all subsequent standardized shipments.

To determine whether gel-pack temperature during packaging affected temperature changes inside the shipping container during transportation, we compared changes in shipping parameters by gel-pack temperature (Supplemental Table 4). We found that the maximum temperature reached inside the container was significantly higher if the gel-pack was warmed to >20–26°C vs. ≤20°C (23.4°C ± 1.5 vs. 21.5°C ± 2.4, respectively; p=0.005). However, there was no significant difference in minimum temperature reached inside the container for warmer vs. cooler gel-packs (15.6°C ± 3.4 vs.14.5°C ± 3.9, respectively; p=0.31).

Several shipping conditions were examined to determine their potential influence on temperature drops within the shipping container (Table 4). Ambient outdoor temperature was shown to be important (overall p=0.04); in particular, a temperature drop inside the shipping container to a value less than 15°C was less likely when the outside city temperature was either −13°C to − 1°C (OR=0.1) or >1°C (OR=0.5) when compared to colder environments of −13°C. Number of Fed-Ex hub-stops during a shipment also was found to be statistically significant; shipments passing through 2 or 3 hubs were more likely to see within container temperature drops <15°C, compared to those only stopping at one hub station (OR=4.5; p=0.04).

Using multivariable logistic regression, minimum outside temperature remained statistically significant (p=0.04), along with borderline significance for the number of Fed-Ex hub-stops (p=0.051).

Optimization of Temperature Control Using Mock Islet Shipments

Because of the large drop and range in temperature values observed during the preliminary shipping evaluation, a series of optimization experiments were carried out to identify the minimum number of gel-packs needed to prevent an undesirable decrease in the climate conditions within the shipping container (Supplemental Figure 2). Use of 6 gel-packs was able to prevent an undesirable temperature drop below 15°C in both cold and warm conditions tested. When 2 different alternating temperature conditions were evaluated using 6 gel-packs, the temperature never fell below 15°C. In all optimization experiments performed, the within-container temperature never exceeded 29°C, which was less than the maximum value tested, i.e. 37°C.

Validation of Standardized Shipping Container

After implementation of the standardized shipping protocol, temperature fluctuations inside the container were monitored from November 1, 2007 to December 31, 2010, during transportation of 1,432 shipments of human islets (Table 5). Using 6 gel-packs, there was no statistically significant difference in number of shipments reaching within-container temperature of ≤15°C (cold-triggered), ≥29°C (heat-activated), or maintained at 15–29°C, across Winter, Spring, Summer, or Fall (p=0.58 by Fishers exact test). Comparing the use of 2 (preliminary shipping evaluation) vs. 6 (standardized shipments) gel-packs in the Winter season, there was a statistically significant improvement in temperature control during shipment (p<0.0001). The percentage of shipments that were cold-triggered was reduced from 36% (n=16) to 13% (n=37), and the percentage of shipments with a stable temperature improved from 64% (n=29) to 87% (n=250).

To analyze our secondary endpoint of islet quality assessment, we compared the 906 non-standardized shipments to the 1,432 standardized shipments (Table 6). Controlling for pre-shipment quality categories (Superior vs. Average), there was a higher percentage of excellent to good post-shipment islet quality ratings using the standardized shipping SOP, compared to shipments in the non-standardized time period (p<0.0001). The percentage of

shipments scored as excellent to good increased by at least 10% after implementation of the standardized shipping protocol.

DISCUSSION

Standardized methods of transporting human islets are needed to preserve the health of preparations during shipment. With the increasing number of long distance basic science and clinical collaborations (4,8,11,22–24,30,32,33,37,38,53), the availability of such protocols is critical to the feasibility of utilizing remote pancreas processing laboratories for clinical transplantation studies (18). Use of different transportation methods, such as commercial airline transporters (33,38), charter jet (18), or ambulance (23,24), makes the widespread adaptation of any single protocol impractical.

This report details the development and validation of a standardized shipping protocol designed to comply with non-clinical classification, packaging, labeling, and documentation regulations on shipping of viable human tissue by the International Air Transport Association (IATA) and the United States Department of Transportation (US DOT). This protocol was used by the ICR Consortium in 1,432 human islet shipments delivered to diabetes investigators via a single commercial airline transporter, and demonstrated improved temperature control compared to 906 shipments using local single center shipping strategies, prior to the availability of a uniform shipping approach.

Our preliminary evaluation of 48 shipments using standardized materials and operating procedures revealed a number of issues that needed to be addressed prior to widespread implementation of a final shipping protocol. First, we observed that packing preparations affected differences in extreme within-container temperatures during shipment. In particular, gel-pack temperatures of ≤20°C vs. >20.0 to 26.0°C yielded as much as a 3°C difference in the mean high and low temperatures. Each package initially contained 2 ambient temperature stabilization gel-packs. Each gel-pack was double-bagged and contained a proprietary solution with a lower melting temperature than any of the individual components within the mixture, i.e. eutectic solution, conferring added thermal capacity and stability at its phase change temperature.

A phase change in each gel-pack, from a solid (frozen) to liquid (melted) state, occurs at 19.6°C±1°C (personal communication with SEBRA). It is therefore likely that when the gel-packs remained in the frozen state, the contents of the container remained cooler in hot and cold conditions, a suggestion consistent with our data. It should be noted, however, that gel-pack temperature alone was not associated with a drop in within-container temperature ≤15°C during shipment. Another factor identified was the number of gel-packs included inside the container, a variable previously highlighted by Rozak and colleagues (43). The SEBRA manual states that ambient temperatures below 10°C or above 38°C may require additional stabilizers. In fact, ambient city temperatures outside the container ranged from -19.4°C to 31.7°C, with 36 of 48 shipments (75%) exposed to lows of <1°C. Temperature highs above 38°C were not observed, as our initial experiments were performed in the winter months only, a potential limitation of this study.

Although other studies have observed temperature changes inside the shipping container during transport (18,19,43), this is the first study we are aware of that attempts to model all of the factors involved. We found that the use of a commercial airline transporter resulted in as many as 3 hub-stops prior to package delivery, and temperature inside the shipping container was 4.5 times more likely to drop ≤15°C if more than one hub-stop was made. This may be explained, in part, by the fact that packages shipped using a commercial airline transporter are placed in a pressurized, but not temperature regulated, cargo carrier (personal

communication with Fed-Ex) and therefore vulnerable to extreme changes in ambient temperature. Indeed, we found that if the lowest departing, arriving, and hub-stop city temperatures were warmer, there was a progressive decline in the odds that a temperature drop to ≤15°C occurred.

Although the mean overall, minimum, and maximum temperatures during shipment (Table 3) were within 1.5°C of the values shown to be acceptable by Ichii and colleagues (18), the maximum change was 18.9°C, similar to the undesirable extreme values reported by Ikemoto (19) and Rozak (43). Moreover, depending on whether using readings from the reusable loggers or disposable indicators, 30% or 36% of all shipments, respectively, reached a temperature low that dropped ≤15°C.

We sought to eliminate temperature drop occurrences falling below 15°C by optimizing the number of gel-packs included inside the standardized shipping container, as it had been previously reported that the use of additional packs stabilized temperature during blood product transport (20). Although the preliminary shipping evaluation called for the use of 2 gel-packs, the final design of the shipping package required experimental testing of this factor to establish the optimal number required for thermal stability. It was determined that a minimum of 6 gel-packs was required to eliminate undesirable temperature drops. Although 8 gel-packs worked equally as well, we choose to use 6 because of the cost savings realized by not including an additional 2 gel-packs per shipment.

Implementation of a standardized islet shipping procedure occurred on November 1, 2007. When we compared islet quality in the 3-year period prior vs. post implementation, we found statistically significant improvements in the “excellent to good” quality ratings, adjusting for pre-shipment purity/viability of the islets (Table 6). Although this suggests that the use of this protocol positively affected islet quality at the receiving laboratory, ratings may have also been attributable to ICR shipping experience over time, such that improvements may have occurred independently of standard materials and operating procedures. While our data cannot rule out this possibility, a number of participating islet laboratories were shipping islets before the creation of the ICR program and thus deemed experienced manufacturers.

The fact that post-shipment islet quality was a qualitative measure poses limitations to the study. Ratings were based on methods not specified or by post-receipt assessment of count, purity, viability, and/or islet usability in laboratory experiments. However, given the large number of investigators receiving human islets, and their laboratory staff, it was not practical to implement uniform quantitative post-shipment islet quality assessment protocols. Although several aspects of the shipping process were standardized for this study, there are other parameters that might also be important in improving the quality of transported pancreatic islets. For example, in this protocol, islets were placed into a tissue culture shipping bag; however, the choice of shipping culture vessel, such as bag, flask, or tube, has been previously shown to influence islet quality (3,18,19). Minimization of thermal fluctuations was achieved passively using gel-packs, but active control devices to regulate temperature and pressure changes during shipping of human pancreatic islets have also been shown to provide excellent thermal stability, with only a potentially nominal increase in the overall weight of the container due to the different phase-change material used (43).

Finally, although we targeted a temperature range that included a midpoint value commonly used during islet culture (22°C), Noguchi and colleagues recently showed that preservation at 4°C may be optimal over culture at 22°C or 37°C (35). Although this work has implications for our study, since we used different media and conditions than those reported

by Noguchi, we were not able to directly test or compare the impact of extreme hypothermic conditions on islet outcome, nor was it within the scope of this investigation to do so. Nonetheless, our standardized shipping protocol can accommodate different temperature ranges by optimization of factors such as packing temperature or number and phase-change status of gel-packs.

Conclusion

Results from 906 pre- vs. 1,432 post-standardized ICR shipments showed a dramatic and significant improvement in the control of environmental shipping factors related to temperature fluctuation during transportation of human islets using a single commercial airline transporter. The ICR-standardized shipping approach has been demonstrated as an easily reproducible, cost-effective, and successful strategy in minimizing the thermal fluctuations experienced by human islets in transit using commercial couriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Standardized Islet Shipping Container Materials Used During Study.

Item[A]	Number Per Shipping Container	Vendor (Location)	Model Number
Expanded polystyrene foam container enclosed in corrugated carton	1	ThermoSafe (Hayward, CA)	355
Ambient temperature stabilizing gel packs	0–8[B]	SEBRA, Haemoentics Corp. (Braintree, MA)	1290
DURASORB underpads	2	COVIDIEN (Mansfield, MA)	1093
Bubble wrap	≥3 layers	ULINE (Waukegan, IL)	S-214
Biodegradable cornstarch peanuts	To capacity	ULINE (Waukegan, IL)	1564
Tempasure plus disposable temperature indicator	1	Tip TEMPerature Products (Burlington, NJ)	TLCSEN 364A
Reusable HOBO temperature data logger[C]	1	Onset (Bourne, MA)	UA-004-64
Reusable HOBO pressure data logger[C]	1	Onset (Bourne, MA)	HPA-0015
Permalife 240mL FEP cell culture bag	1	Origen (Austin, TX)	PL240
CMRL 1066 supplemented media	240mL	MediaTech, Inc. (Manassas, VA)	99-603-CV
Kodak Digital Camera[D]	0	Office Depot (Delray Beach, FL)	C530

[A] Does not include packing or masking tape

[B] Preliminary shipping evaluation (2), optimization experiments (0–8), and final transportation protocol (6)

[C] Software (model #s BCP4.3-ON, BHW-PC) and adapters (model #s USB232, BASE-U-1) were also purchased from Onset

[D] Used to monitor compliance with packing SOP

Table 2
Description of Shipment Data Obtained During Preliminary Shipping Evaluation

Detailed information on temperature and transportation of standardized islet shipping containers was collected for this study.

Parameters of Interest[A]	**No.**[B] **(%)**
Reusable Temperature Data Logger	
Complete data obtained	46 (96%)
Delayed shipment	2 (4%)
Disposable Temperature Indicator	
Complete data obtained	45 (94%)
Indicator not included	2 (4%)
Indicator not set up correctly	1 (2%)
Fed-EX Transportation Information[C]	
Number of hub stops prior to delivery	
1	37 (77%)
2	10 (21%)
3	1 (2%)
Ambient City Temperature[D]	
Complete data obtained	48 (100%)

[A] Total of 9 participating sites, i.e. 8 laboratories and 1 coordinating center

[B] Number of Shipments (No.)

[C] Other variables collected included departure/arrival times and cities, and total miles traveled.

[D] Departing, arriving, and all hub-stop city temperatures collected using data provided by www.weather.com

Table 3

Changes in Shipping Parameters During Transportation of Islet Containers

Data based on preliminary shipping evaluation

	No.[A]	Mean	SD	Median	Min	Max
Temperature within Shipping Container[B]						
Overall (°C)	46	17.5	2.8	18.1	4.7	26.6
Lowest value reached (°C)	46	15.2	3.6	16.4	4.7	18.7
Highest value reached (°C)	46	22.7	2.1	22.9	15.7	26.6
Change range (°C)	46	7.5	3.8	6.8	2.1	18.9
Fastest change rate (°C/minute)	46	0.32	0.24	0.29	0.05	1.29
Ambient (Outside) City Temperature						
Lowest value reached (°C)	48	−4.6	10.3	−6.1	−19.4	16.1
Highest value reached (°C)	48	20.9	4.8	20.6	15.0	31.7
Change range (°C)	48	25.6	12.0	23.1	2.2	48.3
Shipping Time[C]	46	27.3	8.5	25.3	21.7	71.1
On time delivery (20–28 hours)	42	25.1	1.4	25.2	21.7	27.9
Delayed (>28 hours)	4	49.9	17.5	50.0	28.5	71.1
Shipping Distance (miles)	48	1,851	726	2,016	38	2,544

[A] Number of Shipments (No.) with data

[B] Monitored using reusable temperature data loggers

[C] Defined from the estimated time of packing, one hour prior to Fed-Ex pickup, to arrival at the data coordinating center in Duarte, CA.

Table 4

Univariate Logistic Regression Analysis of Shipping Conditions Associated with a Temperature Drop to ≤15°C Inside the Container

Data summarize the preliminary shipping evaluation experience.

	OR[A] (95% CI)	p-value
Gel-pack temperature at packing (°C)		
>20–26 (n=29)	--	0.35
≤20 (n=17)	1.8 (0.5, 6.6)	
Lowest outside city temperature (°C)		
<−13 (n=12)	--	0.04
−13 to1 (n=24)	0.1 (0.0, 0.7)	
≥1 (n=10)	0.5 (0.1, 2.6)	
Highest outside city temperature (°C)		
< 17 (n=10)	--	0.6
17–22 (n=27)	2.4 (0.5, 117.7)	
≥ 22 (n=9)	2.0 (0.3, 19.2)	
Fed-Ex hubs used per shipment		
1 (n=36)	--	0.04
2 or3 (n=10)	4.5 (1.1, 21.3)	
Shipping distance (miles)		
<1500 (n=5)	--	0.47
1500–2300 (n=33)	0.9 (0.1, 7.4)	
≥2300 (n=11)	0.3 (0.0, 3.7)	
Shipping time (hours)		
<24 (n=10)	--	0.47
24–28 (n=32)	0.5 (0.1, 2.3)	
>28 (n=4)	1.3 (0.1, 14.8)	

[A]Odds ratios, confidence intervals and p-values were calculated using univariate logistic regression. Dashes indicate baseline category.

Table 5

Temperature Control in 1,432 Shipments of Human Pancreatic Islets

The temperature inside the islet shipping container was the primary outcome variable and evaluated using a standardized shipping protocol, differing only in the number of ambient temperature gel-packs.

Temperature During Shipment (°C)[A]	Standardized Shipments[B]										p-value[C]
	2 Gel-packs		6 Gel-packs								
	Winter		Winter		Spring		Summer		Fall		
	n	%	n	%	n	%	n	%	n	%	
≤15 °C reached	16	35.6	37	12.8	47	11.4	35	9.3	47	13.3	<0.0001
Always 15–29 °C	29	64.4	250	86.5	363	87.9	336	89.4	305	86.2	
≥29 °C reached	0	0.0	2	0.7	3	0.7	5	1.3	2	0.5	
TOTALS	**45**	**100**	**289**	**100**	**413**	**100**	**376**	**100**	**354**	**100**	

[A] Monitored using disposable temperature indicators

[B] Shipments were binned into seasons, depending on package pick-up date. Seasons defined using equinox and solstice dates, for 2007–2010, taken from United States Naval Observatory website at: http://www.usno.navy.mil/USNO/astronomical-applications/data-services/earth-seasons

[C] Significant difference between use of 2 versus 6 gel-packs in the Winter, with a Bonferroni-corrected nominal α=0.025, by Fisher's exact test; see text for additional detail.

Table 6

Islet Quality Ratings in 2,338 Shipments of Human Pancreatic Islets

Post-shipment islet quality was a secondary outcome variable and compared pre and post implementation of a standardized shipping protocol for shipments made by the ICR Basic Science Human Islet Distribution Program.

Pre-Shipment Islet Quality[A]	Shipping Condition	Post-Shipment End User Assessment[B]				p-value[C]
		Fair to Poor		Excellent to Good		
		n	%	n	%	
Superior	No Standard SOP	40	18.9	172	81.1	<0.0001
	Standardized SOP	36	7.4	452	92.6	
Average	No Standard SOP	136	19.6	558	80.4	
	Standardized SOP	92	9.7	852	90.3	

[A] Determined by the islet manufacturing facility prior to transportation; Superior (≥85% purity & >90%viability), Average (<85% purity or ≤90% viability or both).

[B] A qualitative assessment performed post-shipment by the islet-receiving laboratory; additional details in materials and methods.

[C] Determined using the Cochran-Mantel-Haenszel test statistic. Prior to that, the Breslow-Day test for homogeneity of proportions was used to examine the dependence of post-receipt ratings on pre-shipment islet quality (p=0.3679).

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