

## Detection of Microbial Contamination During Human Islet Isolation

Tatsuya Kin, Shawn Rosichuk, A. M. James Shapiro, and Jonathan R. T. Lakey

Clinical Islet Transplant Program, University of Alberta and Capital Health Authority, Edmonton, Alberta, Canada

Current good manufacturing practice (cGMP) islet processing facilities provide an ultraclean environment for the safe production of clinical grade islets for transplantation into immunosuppressed diabetic recipients. The objective of this study was to monitor the rate of microbial contamination in islet products after implementation of good manufacturing practice conditions. Fluid samples for microbial contamination were collected at the following steps: from the pancreas transport solution upon arrival of the organ ( $n = 157$ ), after surface decontamination of the pancreas with antiseptic agents ( $n = 89$ ), from islet supernatant at the end of the isolation ( $n = 104$ ), and from islet supernatant as a final transplantable product after culture ( $n = 53$ ). Bacterial, fungal, and mycoplasma cultures were conducted for 2, 2, and 3 weeks, respectively. Microbial contamination was detected in 31% of transport solution. The contamination was not associated with the presence of the duodenum during the preservation, cold ischemia time, or procurement team (local vs. distant). Surface decontamination of the pancreas resulted in clearance of 92% of the microbial contamination. Six preparations at the end of the isolation revealed microbial growth. All were de novo contamination during the processing. Fifty-three preparations that met our release criteria in terms of product sterility were transplanted into type 1 diabetic patients. In two instances, positive culture of the islet preparation was reported after transplantation had occurred. No patient showed any clinical findings suggestive of infection or any radiological abnormalities suggestive of abscess; a single dose of antibiotic coverage was given routinely to recipients prior to islet infusion. Although transport solution carries a high risk of microbial contamination, most contaminants become undetectable during islet processing. Microbial contamination in final products is rare, but de novo contamination still occurs during processing even under cGMP conditions.

**Key words:** Microbial contamination; Quality control; Islet isolation; Antibiotics

### INTRODUCTION

Islet transplantation into patients with type 1 diabetes has now become a viable treatment for this devastating disease (6,9,13,15). To ensure safety and quality of human islet products for transplantation, a policy of strict quality control of the product is essential. The controls should include islet yield, purity, tissue volume, viability, and follow-up of microbiological sterility. Because the patients are immunosuppressed, microbial contamination of islet products may threaten islet recipients even under antibiotic treatment (17). National regulation boards are now imposing specific requirements that a transplantable suspension must meet in order to be safely transplanted (2,4,5).

Several studies have been published analyzing the microbial contamination rate of islet products. The investigators found a microbial contamination rate of 0–16% (1,3,8,14). Contamination of the islet product can

occur at several stages during harvesting of the gland, islet isolation processing, and culturing. We studied the results of microbial cultures prospectively performed in 157 pancreata for islet isolation. Samples for cultures were taken at different time points during processing. The objective of the study was to monitor the rate of microbiological contamination of islet products under good manufacturing practice conditions. We also investigated association of the presence of the duodenum during the storage with contamination because the duodenum can be the source of contamination.

### MATERIALS AND METHODS

#### *Procurement and Preservation of the Pancreas*

Between April 2003 and March 2005, 157 pancreata were procured from brain-dead donors after aortic flush with cold University of Wisconsin solution (UW) and shipped to our islet isolation laboratory. We asked both local and distant organ procurement teams to procure

the pancreas en block with the duodenum after dividing the duodenum with a linear cutting stapler. Ninety pancreata along with the duodenum placed in cold UW were transported to the laboratory. In the remaining 67 cases, the duodenum was removed on the back table at the operation room, then the pancreas alone was preserved in the perfluorocarbon and UW two-layer solution ( $n = 64$ ) or in UW ( $n = 3$ ) prior to islet isolation. In six pancreata preserved in cold static UW, the organ along with the duodenum was further preserved in the laboratory by using a hypothermic machine perfusion (LifePort™; Organ Recovery Systems, Des Plaines, IL) for up to 24 h.

#### *Surface Decontamination of the Pancreas and Islet Isolation*

Upon arrival of the pancreas, the duodenum was dissected from the pancreas. The pancreas was washed with 1.5% povidone-iodine (Betadine, Purdue Pharma, Ontario, Canada) solution, then with 150 ml of Hanks' balanced salt solution (HBSS) containing 1 g of cefazoline (Novopharm, Toronto, Canada) and 100 mg of amphotericin B (Bristol-Myers Squibb Canada Inc., Montreal, Canada), and finally rinsed with HBSS. The pancreas was transferred on a cut-down tray containing 500 ml of Euro-Collins solution for the insertion of catheters into the main duct. The gland was perfused with enzyme thorough the catheters and then transferred to a digestion device for mechanical and enzymatic digestion. Pancreatic digest containing endocrine and exocrine tissue was purified by continuous density gradients using a refrigerated COBE 2991 cell processor (COBE Laboratories, Inc., Lakewood, CO). During the process, islet tissue was washed with wash solution (Mediatech Inc., Herndon, VI) supplemented with 4 mg/L Ciprofloxacin (Bayer Inc., Toronto, Canada). A total amount of wash solution used was typically 6 L for the process. The islet products were processed under environmental conditions International Standards Organization (ISO) Class 5 in Class II biological safety cabinets placed in a clean room with background condition ISO Class 7.

#### *Sampling*

Fluid samples (approximately 5 ml) for microbial contamination were collected at the following steps: step 1, from the pancreas transport solution (UW) upon arrival of the organ ( $n = 157$ ); step 2, from the Euro-Collins solution on the tray after insertion of the catheters ( $n = 89$ ); step 3, from islet supernatant at the end of the isolation procedure ( $n = 104$ ); step 4, from islet supernatant as a final transplantable product after culture ( $n = 53$ ). Prior to transplantation, islets were cultured for  $22.5 \pm 2.2$  h (mean  $\pm$  SEM) in CMRL-based medium at 22°C. Initial recipients received antibiotics prophylaxis con-

sisting of 500 mg of vancomycin and 500 mg of imipenem, but our routine practice since 2003 has been to give cefotaxime 2 g IV on call to the procedure, with the addition of metronidazole 500 mg IV if the subject has had prior upper abdominal surgery. In six cases of the machine perfusion, perfusate samples were collected every 3 h for the first 12 h and at the end of perfusion preservation. Bacterial, fungal, and mycoplasma cultures were conducted for 2, 2, and 3 weeks, respectively.

#### *Statistical Analysis*

Data are presented as proportions for categorical variables. The incidence of culture-positive cases was compared by the chi-square test. A univariate logistic regression analysis was used to examine the relationship between UW contamination and cold ischemia time. Levels of statistical significance were set at  $p < 0.05$ . Data analysis was performed with SPSS statistical software version 11.5 (SPSS, Inc., Chicago, IL).

## RESULTS

Of 157 samples at step 1, 48 cases showed positive microbial growth. The most frequent microorganism identified was coagulase-negative *Staphylococcus* in 17, followed by *Propionibacterium* in 10, and *Klebsiella pneumoniae* in 7. Ninety of 157 pancreata were transported along with the duodenum. Among them the stumps of the duodenum was left open ( $n = 2$ ) or not properly sealed with a stapler ( $n = 1$ ) in three cases; therefore, these UW were all contaminated with bacteria. In one pancreas procured from a donor with having a perforation in the bowel, the UW was also contaminated with bacteria. In the remaining 86 pancreata with completely sealed duodenal stumps, 29 (33.7%) revealed positive culture results. This figure was similar with contamination rate of the UW when the duodenum was removed before the preservation (22.4%, 15 of 67,  $p = 0.124$ ) (Table 1). Cold ischemia time (defined as the time from the cross-clamping to the step 1) did not associate with microbial contamination of UW with the presence of the

**Table 1.** Microbial Contamination of Transport Solution

Transport Solution	Duodenum Attachment	
	No	Yes
Culture negative	52	57
Culture positive	15	29

Contamination was present in 33.7% and 22.4% of transport solution with and without presence of the duodenum, respectively ( $p = 0.124$ ; chi-square test).

duodenum (Table 2). Procurement team (local vs. distant) was not associated with contamination rate of UW (32.4% in local vs. 30.0% in distance,  $p = 0.78$ ). In six pancreata applied for the machine perfusion, all samples at step 1 were not contaminated with microorganism. During the machine perfusion for up to 24 h, all samples of perfusate had negative culture results.

Of 89 samples at step 2, 13 cases showed positive microbial testing. Eight cases of 13 were de novo contamination during the processing. In the remaining five samples the microorganisms were identical with those isolated at step 1. Of these, three had severely contaminated UW because of leakage of duodenal contents, as mentioned above. Among 76 samples with negative growth at step 2, UW was contaminated in 56 cases. Thus, decontamination of the pancreas resulted in clearance of the microbial contamination in 91.8% (56 of 61).

Of 104 samples at step 3, six (5.8%) revealed positive microbial growth consisting of *Propionibacterium* in three, *Viridans group strep* in one, *Acinetobacter Iwoffii* in one, *Mycoplasma* in one. All six were de novo contamination during the islet processing. The preparations isolated from the contaminated organs for duodenum leakage revealed negative growth. Five preparations were not used for transplant because of low yield in islets. One was transplanted but the final sample at step 4 revealed negative growth.

During the study periods, 53 islet preparations were transplanted into type 1 diabetic patients. All 53 preparations met our release criteria in terms of product sterility (negative gram staining and less than 5 endotoxin units/kg body weight); however, two preparations revealed positive microbial growth after transplantation (one with *Streptococcus* species and *Rothia* species and another with *Mycoplasma* species). Again, both were de novo contamination. All patients including the two receiving contaminated products did not show any clinical findings suggestive of infection after transplantation.

There was a pancreas procured from a donor who showed positive bacterial culture (methicillin-resistant *Staphylococcus aureus*) in the blood sample. Although

the islet preparation was not used for transplant, all three samples during the process did not reveal any microorganism growth.

## DISCUSSION

We found that the rate of microbial contamination was highest in the transport solution surrounding the pancreas organ during transport, which is in accordance with previous studies (1,3,8,14). The sources of contamination are either endogenous or exogenous, or both. One of the endogenous sources can be the duodenum, which is generally procured en block with the pancreas. In this study we examined whether the presence of the duodenum in the transport solution contributes to microbial contamination. Although there were three cases of contamination of the transport solution with the presence of the disrupted duodenum, the incidence of contamination of transport solutions with the presence of the "intact duodenum" did not differ from those without. Longer storage time with the presence of the duodenum could give more chance of contamination in transport solution. However, we did not find that cold ischemia time associated with contamination of the transport solution in the presence of the duodenum. Furthermore, sterility of perfusate was confirmed for up to 24 h in the machine perfusion of the pancreas along with the duodenum. Taken together, storage of the pancreas with the duodenum would not increase the risk of contamination when the stumps are staple closed appropriately. It should be noted that the high rate of bacterial contamination in the salvaged blood has been reported in nonabdominal surgery: 67% in cardiovascular surgery (16) and 47% in neurosurgery (7).

The three cases with the leakage of the duodenal contents had the identical microorganism in the transport solutions with those identified in the samples after decontamination of the pancreas, suggesting that our decontamination procedure is not effective enough to clear a notable microbial contamination. However, decontamination of the pancreas resulted in clearance of 92% of the microbial contamination. We did not ascertain whether our antiseptic agents contributed to this reduction. In a recent study, a similar decontamination protocol to ours did not result in a significant reduction in microbial contamination rate when compared to historical controls using an alternative method without antiseptic agents (1). Povidone-iodine is known to be effective against both aerobic and anaerobic bacteria and fungi, protozoa, and viruses. Its antimicrobial effect occurs within seconds of contact (12). However, Olson and his colleague reported that decontamination efficacy of 10% povidone-iodine was equivalent to amino acid solution in the study of small bowel preservation (10). Further studies are needed to

**Table 2.** Univariate Association Between Cold Ischemia Time and Microbial Contamination in Transport Medium With the Presence of the Duodenum

Variable	Coefficient	95%		$p$
		Odds Ratio	Confidence Interval	
Cold ischemia time	0.042	1.043	0.927–1.173	0.485

Odds ratio: >1, positive correlation with microbial contamination.

investigate the effect of antiseptic agents on decontamination of the pancreas.

Although the rate of microbial contamination of transport solution was considerably high, most contaminants were washed or diluted out during the islet processing, which is in agreement with previous studies (1,3,8,14). The important finding in this study is that all positive microbial growths in samples obtained at the end of processing (steps 3 and 4) were de novo contaminations during the islet processing. It is difficult to ascertain which procedures during isolation are responsible for de novo contaminations. But we believe that the sterile technique and environmental controls in an islet isolation facility are of paramount importance. In a previous study (8), we reported that microbial contamination rate at postisolation step was 4.3% (3 of 70), which is comparable to the figure in this study of 5.8% (6 of 104). The previous study was done before implementation of GMP conditions whereas this study was conducted under clean room conditions. This may lead to the question of how critically important ultraclean room conditions are in a cell processing facility. However, it should be noted that in our previous study microbial culture was performed for up to only 48 h for bacterial detection alone while in the current study culture duration was up to 3 weeks and fungal and mycoplasma cultures were conducted as well. Ritter et al. found that bacterial contamination rate was reduced in cellular-based products after implementation of clean room conditions (11).

The sterile technique during islet isolation continues to be of paramount importance in line with the continual improvement in islet transplantation safety. In our series, there were two cases of contaminated islet preparation transplanted. Fortunately, the transplantation did not result in serious infectious complications in an immunosuppressed patient with antibiotic prophylaxis. Nonetheless, infusion of frankly infected islet preparations could be seriously deleterious to an immunosuppressed patient and must therefore be avoided.

**ACKNOWLEDGMENTS:** *This study was supported by a Clinical Center Grant from the Juvenile Diabetes Research Foundation. J.R.T.L. is a recipient of scholarships from the Canadian Diabetes Association and the Alberta Heritage Foundation for Medical Research (AHFMR). A.M.J.S. holds a scholarship with the AHFMR, and has a Canadian Institutes for Health Research chair in transplantation. We acknowledge the Human Organ Procurement Exchange program of Alberta for assistance in identifying and recovering human cadaveric pancreata, and the technical staff members of the Clinical Islet Isolation Laboratory, University of Alberta. Additional support for this study was provided through generous donations from the Roberts Family, the North American Foundation for the Cure of Diabetes, the Alberta Building Trades, and from the Diabetes Research Institute Foundation of Canada.*

## REFERENCES

1. Bucher, P.; Oberholzer, J.; Bosco, D.; Mathe, Z.; Toso, C.; Buhler, L. H.; Berney, T.; Morel, P. Microbial surveillance during human pancreatic islet isolation. *Transpl. Int.* 18:584–589; 2005.
2. Burger, S. R. Current regulatory issues in cell and tissue therapy. *Cytotherapy* 5:289–298; 2003.
3. Carroll, P. B.; Ricordi, C.; Fontes, P.; Rilo, H. R.; Phipps, J.; Tzakis, A. G.; Fung, J. J.; Starzl, T. E. Microbiologic surveillance as part of human islet transplantation: Results of the first 26 patients. *Transplant. Proc.* 24:2798–2799; 1992.
4. Galbraith, D. N. Regulatory and microbiological safety issues surrounding cell and tissue-engineering products. *Biotechnol. Appl. Biochem.* 40:35–39; 2004.
5. Giordano, R.; Lazzari, L.; Rebull, P. Clinical grade cell manipulation. *Vox Sang.* 87:65–72; 2004.
6. Hering, B. J.; Kandaswamy, R.; Harmon, J. V.; Ansit, J. D.; Clemmings, S. M.; Sakai, T.; Paraskevas, S.; Eckman, P. M.; Sageshima, J.; Nakano, M.; Sawada, T.; Matsumoto, I.; Zhang, H. J.; Sutherland, D. E.; Bluestone, J. A. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am. J. Transplant.* 4:390–401; 2004.
7. Kudo, H.; Fujita, H.; Hanada, Y.; Hayami, H.; Kondoh, T.; Kohmura, E. Cytological and bacteriological studies of intraoperative autologous blood in neurosurgery. *Surg. Neurol.* 62:195–200; 2004.
8. Lakey, J. R.; Rajotte, R. V.; Warnock, G. L. Microbial surveillance of human islet isolation, in vitro culture, and cryopreservation. *Clin. Invest. Med.* 18:168–176; 1995.
9. Markmann, J. F.; Deng, S.; Huang, X.; Desai, N. M.; Velidedeoglu, E. H.; Lui, C.; Frank, A.; Markmann, E.; Palanjian, M.; Brayman, K.; Wolf, B.; Bell, E.; Vitamanuk, M.; Doliba, N.; Matschinsky, F.; Barker, C. F.; Naji, A. Insulin independence following isolated islet transplantation and single islet infusions. *Ann. Surg.* 237:741–749; 2003.
10. Olson, D. W.; Kadota, S.; Cornish, A.; Madsen, K. L.; Zeng, J.; Jewell, L. D.; Bigam, D. L.; Churchill, T. A. Intestinal decontamination using povidone-iodine compromises small bowel storage quality. *Transplantation* 75: 1460–1462; 2003.
11. Ritter, M.; Schwedler, J.; Beyer, J.; Movassaghi, K.; Muters, R.; Neubauer, A.; Schwella, N. Bacterial contamination of ex vivo processed PBPC products under clean room conditions. *Transfusion* 43:1587–1595; 2003.
12. Rodeheaver, G.; Bellamy, W.; Kody, M.; Spatafora, G.; Fitton, L.; Leyden, K.; Edlich, R. Bactericidal activity and toxicity of iodine-containing solutions in wounds. *Arch. Surg.* 117:181–186; 1982.
13. Ryan, E. A.; Lakey, J. R.; Rajotte, R. V.; Korbitt, G. S.; Kin, T.; Imes, S.; Rabinovitch, A.; Elliott, J. F.; Bigam, D.; Kneteman, N. M.; Warnock, G. L.; Larsen, I.; Shapiro, A. M. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50: 710–719; 2001.
14. Sharp, D. W.; Lacy, P. E.; McLearn, M.; Longwith, J.; Olack, B. The bioburden of 590 consecutive human pancreata for islet transplant research. *Transplant. Proc.* 24: 974–975; 1992.
15. Shapiro, A. M.; Lakey, J. R.; Ryan, E. A.; Korbitt, G. S.; Toth, E.; Warnock, G. L.; Kneteman, N. M.; Rajotte,

- R. V. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* 343:230–238; 2000.
16. Shindo, S.; Matsumoto, H.; Kubota, K.; Kojima, A.; Matsumoto, M. Temporary bacteremia due to intraoperative blood salvage during cardiovascular surgery. *Am. J. Surg.* 188:237–239; 2004.
17. Taylor, G. D.; Kirkland, T.; Lakey, J.; Rajotte, R.; Warnock, G. L. Bacteremia due to transplantation of contaminated cryopreserved pancreatic islets. *Cell Transplant.* 3: 103–106; 1994.