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Microbial surveillance during human pancreatic islet isolation

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Summary

The aim of the study was to investigate microbiological contamination rate during human pancreatic islet isolation. Between 1996 and 2002, pancreas preservation media and postpurification islet preparations were screened for microbiological contamination. After arrival in the laboratory, pancreata were washed prior to enzyme perfusion with either Hank's balanced salt solution (Group I, $n = 170$, 1996 to 2001) or decontaminated with polyvidonum-iodine, cefazoline, and amphotericine B (Group II, $n = 45$, 2001 to 2002). Microbiological contamination of preservation media was observed in 56% and 84% for Groups I and II, respectively. Analysis of contaminants revealed 74% Gram-positive, 21% Gram-negative bacteria and 5% fungi. Duration of transport had an influence on the rate of contamination ($P < 0.05$). After islet isolation, Group I presented microbial contamination of 16 islet preparations (9.4%) [i.e. Gram-positive bacteria ($n = 10$), Gram-negative bacteria ($n = 4$), and fungi ($n = 2$)]. In Group II, only 2 islet preparations (4.4%) presented microbial contamination. Microbial contamination during pancreas procurement occurs frequently. Most microorganisms are eliminated during islet isolation, and de novo contaminations during islet isolation are rare. Pancreas decontamination reduces the risk of infection of the final islet preparation.

Introduction

Islet transplantation has long been studied as an alternative treatment of diabetes. Since the last breakthrough in this field, i.e. the successful clinical trial of islet transplantation for type I diabetic patients by the Edmonton group, much attention has been given to the treatment of diabetes by islet transplantation [1–3]. Pancreatic islet isolation and transplantation are likely to expand around the world. Entering clinical routine, the islet isolation process has to meet appropriate safety and quality requirements. Among several factors that should be monitored to ensure the safety and quality of human islet transplantation, sterility of the islet preparation is of utmost importance, as transmissions of pathogens may have deleterious consequences in immunosuppressed hosts [4]. For this reason, we started to trace microbial contamination of the harvested pancreata and of islet preparations since 1996. This study reports the prospective microbial surveillance of pancreas preservation media and of postpurification islet preparations of the last 215 consecutive human islet isolations performed at the University of Geneva.

Materials and methods

Microbiological samples

All human pancreas islet isolations from November 1996 to July 2002, for which the purification steps were completed, were included in the study. During the study period, three pancreata isolation were not included. For all these three, isolation process was not conducted after unsuccessful organ digestion. For each of these human islet isolations, microbiological samples were collected

prospectively for aerobic and anaerobic microbial culture. Of the solutions collected, 10 ml was injected in BACTEC Plus aerobic/F, 10 ml in BACTEC Lytic/10 Anaerobic/F and 10 ml in BACTEC Myco/F-lytic (Becton Dickinson, Sparks, MD, USA) blood cultures flasks. The first microbial samples were taken from the pancreas transport medium just after the opening of the sterile pancreas packing in the isolation laboratory (sterility class 10 000 rooms). The second microbial samples were taken from the gradient solution after the COBE 2991 islet purification, within a sterility class 100 hood (Flufrance VLF 200, Vaulorin, France). For gram stain of final islet preparation we collected 20 ml of final islet preparation, which were processed according to the recommendations provided in the *Manual of Clinical Microbiology* [5]. Microbiological samples were cultured for at least 5 days at 35 °C, using standard procedure, and when positive, the microbial organisms were characterized based on the recommendations provided in the *Manual of Clinical Microbiology* [5].

Pancreas harvesting and transport

Human pancreata were harvested from multiple organ donors at several hospitals in Switzerland, and among the GRAGIL network [6] including the University Hospitals of Besançon, Grenoble, Lyon, Nancy and Strasbourg. The pancreata were removed using standard aseptic surgical techniques and sent to our islet isolation laboratory. pancreata were harvested en bloc with the duodenum, after the duodenum was rinsed with 500 ml of 5% polyvidonum-iodine solution through a nasogastric tube. Transection of the duodenum was performed with disposable staplers. Thereafter, the pancreata were immerged in chilled University of Wisconsin solution without antibiotic addition, and packed under aseptic conditions into, at least, two sterile plastic bags. The pancreas was shipped in an icebox to the islet core facilities at the University of Geneva.

Pancreas processing

Pancreas isolations were performed aseptically in a sterility class 10 000 particles room with two, class 100 particles hoods. Solutions are tested for microbacterial and endotoxin contamination before use for human islet isolation program. From November 1996 to October 2001, the pancreata ($n = 170$) were washed with cold Hank's balanced salt solution (HBSS) before being prepared for collagenase injection under aseptic conditions (Group I). From November 2001 to July 2002, the pancreata ($n = 45$) were recovered from the transport package and decontaminated through immersion in three antimicrobial bathes according to the Edmonton and ITN isolation procedure (Group II). The first bath contained 5% polyvidonum-iodine (Betadine®, Mundipharma, Basel, Switzerland), the second cefazoline (1 g in 150 ml of HBSS; Kefzol®, Lilly, Vernier, Switzerland) and amphotericine B (100 mg in 150 ml of HBSS; Fungizon®, Bristol-Myers Squibb, Baar, Switzerland), and the third bath cold HBSS solution. The pancreas isolation procedure was basically a two steps process, with collagenase digestion at first, followed by purification of the digest with a density gradient centrifugation [7,8]. After the pancreas was cleaned from the surrounding fat and fibrotic tissue, a collagenase solution (Collagenase type P Boeringher–Mannheim, Germany, until December 1997, thereafter, Liberase-HI, Roche, Basel, Switzerland) was injected into the main duct, and the pancreas digestion was performed as a digestion-filtration step. Purification was performed using a COBE 2991 cell processor (Cobe, Lakewood, CO, USA) either with discontinuous Ficoll gradients (Sigma, St Louis, MO, USA), or with continuous Ficoll gradients (Biochrom KG, Berlin, Germany). Penicillin and streptomycin (Roche GmbH, Mannheim, Germany) were added both at a concentration of 56 U/ml to the solutions used during islet isolation since the beginning of the study.

Statistical analysis

Statistical analyses were performed with In Stat software (GraphPad InStat version 3.00 for Windows 95; GraphPad Software Inc, San Diego, CA, USA) on a personal computer. Student's *t*-test or ANOVA test for continuous variables were used, and the χ^2 test or Fisher's exact test were used for categorical variables. A P-value <0.05 was considered as statistically significant.

Results

From November 1996 to July 2002, 215 human pancreata were processed to final islet preparations, from which accurate microbiologic data were available. In Group I, 170 pancreata were processed without organ decontamination from November 1996 to October 2001. In Group II, 45 pancreata were decontaminated prior to start the islet isolation process from November 2001 to July 2002.

Contamination of transport medium

Pancreas transport medium was contaminated in 62% (133 cases) of all 215 procured organs. Among the first 170 pancreata (Group 1), contamination rate was 56% (95 cases) and among the last 45 (Group 2, the rate was 84% (38 cases). In order to understand the high rate of contamination of transport medium, we further analyzed the incidence of contamination according to each

procurement center, and to procurement and transport time (cold ischemia). There was no statistical difference in the prevalence of microbial contamination of the pancreas transport medium between the various procurement centers. The procurement duration was defined as the time between aortic cross clamping to time of completion of pancreatectomy. Comparison of procurement duration of the organs with a cut-off of 30 min, the contamination rates were 50% (<30 min) and 63% (>30 min) ($P = 0.089$). Comparison of cold ischemia duration of organs with a cut-off of 4 h, the rates of contamination were 43% (<4 h) and 60% (>4 h) ($P = 0.049$, Fig. 1).

A single type of microorganism was present in the majority of cases (69%), two and three types of microorganisms were found in 26% and 4% of cases, respectively. Microorganisms were Gram-positive, Gram-negative bacteria and fungi in 74%, 21% and 5% of cases, respectively. Among the contaminated transport media, we isolated a total of 128 different microorganisms species (most common are shown in Fig. 2). Staphylococcal species were found in 51% of cases, streptococcal species in 10%, *Escherichia coli* in 10%, *Propionibacterium* species and *Candida albicans* in 6%, respectively. Other microorganisms were cultivated with lower frequencies and included: *Enterobacter* (5%), *Corynebacterium* (2%), *Klebsiella* (2%), *Citrobacter* (2%), *Pseudomonas* (1%), *Neisseria* (1%), *Serratia* (1%), *Actinomycetes* (1%) and *Haemophilus* (1%).

Contamination of final islet preparations

Microbial screening results of islet preparations were analyzed separately for Groups I and II, in order to evaluate the role of pancreas decontamination. In Group I, microbiological surveillance of islet preparations after purification revealed 16 (9.4%) contaminated preparations. None of these contaminated preparations was infected with more than one microorganism species. Among the 16 contaminated islet preparations, the majority (63%) was infected by Gram-positive organisms, followed by Gram-negative organisms (25%) and fungi (13%). The most common microorganisms cultured from postpurification preparation were Staphylococcal species, *Acinetobacter* species and *Candida albicans* which were cultured 5 (31%), 3 (19%) and 2 (13%) times respectively (Fig. 3). Other microorganisms like Streptococcal species (6%), *Escherichia* (6%), *Propionibacterium* (6%), *Enterobacter* (6%), *Corynebacterium* (6%), *Lactobacillus* (6%) and *Haemophilus* (6%) were cultivated at one instance each.

Among the pancreata processed in Group I, the islet isolation permitted clearance of initial microbial contamination in 83 of 95 pancreata (87.4%). In the remaining 12 pancreata, seven islet preparations were finally infected by the same microorganism as detected initially on the

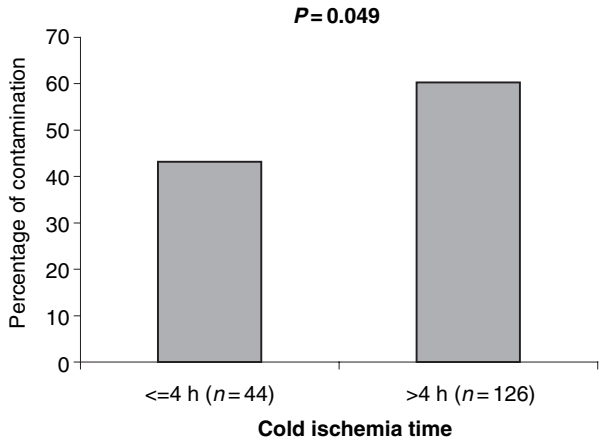

Figure 1 Contamination ratio of pancreas transport medium according to cold ischemia duration. Data on cold ischemia duration were available for 170 pancreata. Contamination was present in 43% of pancreata with <4 h of cold ischemia and in 60% of pancreata with >4 h of cold ischemia ($P = 0.049$; χ^2-test).

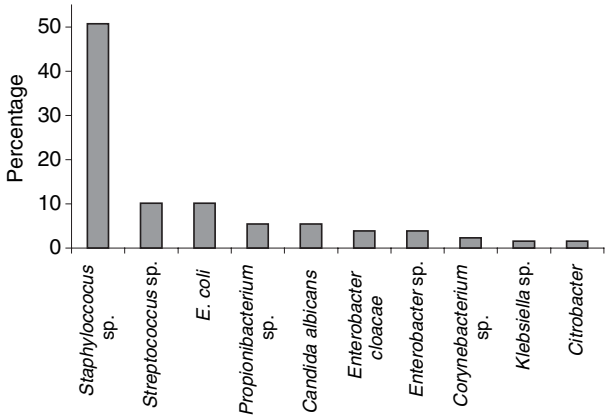

Figure 2 Microbial species cultured from 133 pancreata transport media.

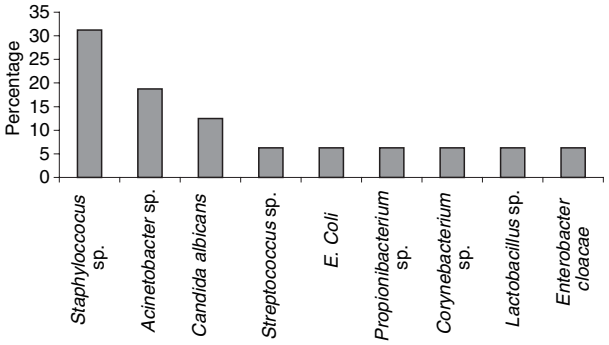

Figure 3 Microbial species cultured from 16 postpurification islet preparations presenting contamination in Group I.

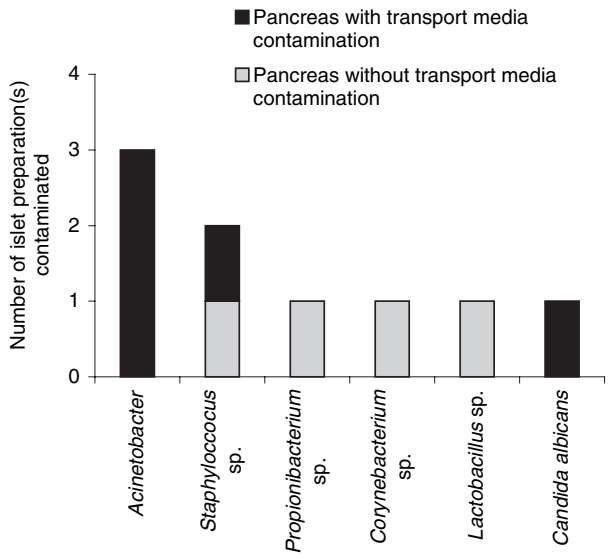

Figure 4 *De novo* microbial species contamination during islet isolation process.

transport medium; however, five final contaminants of islet preparations were organisms not detected initially on transport media and represent contamination during the isolation procedure (Fig. 4). Among the 75 sterile pancreata (with no contamination in transport medium), four (5.3%) islet preparations were contaminated de novo during the islet isolation procedure (Fig. 4). Among the de novo contaminants, the most frequent were *Acinetobacter* (25%) in three instances and staphyloccocal species (8%) in two instances.

In Group II, pancreas decontamination was routinely performed after arrival at our laboratory and prior to islet isolation. Microbiological surveillance of the last 45 postpurification islet preparations revealed 2 (4.4%) contaminated preparations. In both cases, the transport medium was already contaminated with the same bacterial species. The islet isolation procedure, including pancreas decontamination, permitted clearance of the microbial contamination in 36 of the 38 pancreata (94.7%). The rate of islet preparation contamination was reduced by this decontamination from 9.4% to 4.4%; however, these results were not statistically significant ($P > 0.4$).

Human islet transplantation

During the study period, a total of 56 islet preparations were transplanted by intraportal injection to type I diabetic patients (Group I, $n = 41$; Group II, $n = 15$). All transplantations were associated with graft function. All these preparations were negative for microbial pathogen contamination on gram testing, which was confirmed later by culture. During the study period one preparation suitable for transplantation according to islet yields and quality was discarded for infusion because of microbial contamination. This contamination was discovered before infusion by gram staining and confirmed later by culture. None of the transplanted patients experienced any infectious complications related to the islet transplantation. All patients received prophylactic antibiotic therapy.

Discussion

In the present study, we analyzed the rate of microbiological contamination of donor pancreata and postpurification islet preparations. The presented data show that there was a high incidence of microbiological contamination during organ procurement from cadaveric donors and that the islet isolation process could eliminate most of these contaminants. Decontamination of the donor pancreas before the islet isolation process could further reduce microbiological contamination of the final islet preparation.

As islet transplantation is emerging as a new treatment for type I diabetic patients [3,9–11], monitoring of microbial contamination of islets during isolation and purification procedures is an important task for future clinical trials [4,12,13–16], and may prevent disease transmission or at least documentation of the source and early identification of pathogens.

The incidence of transport medium contamination has been previously investigated for various organs. For kidney grafts, the rate of contamination was reported to be between 2% and 23% [14,17–20]. For pancreas grafts, the contamination was higher and varied between 19% and 68% [21–23]. In our study, the rate of pancreas transport medium contamination was 56% and 84% for Groups I and II, respectively. The higher microbiological contamination rate of pancreata, as compared to kidneys, may be related to the presence of the duodenum, which is in general preserved. The differences in the rate of pancreas transport medium contamination, reported in the literature and encountered among the different centers of our study, could be explained by the use of different procurement techniques, such as the use of duodenal decontamination with iodine solutions [23]. Moreover, different attitudes in antibiotic prophylaxis in intensive care units and administration of proton-inhibitors for prevention of stress ulcers, may influence the rate of bacterial and fungal colonization of the upper gastro-intestinal tract in donors and subsequently modify the rate of pancreas graft contamination [24,25].

Our laboratory received pancreata from various hospitals involving a high number of intensive care and surgical teams, and we could not observe any statistical

difference in the rate of contamination among these centers. However, the higher rate of contamination of transport media in Group II is worrisome and might be related to an aging and more morbid donor population, as observed as a global trend in transplantation [26]. However, this was not the case in our study. If donor maintenance in intensive care unit has been modified and could have influenced this results should be investigated in the future.

We observed a trend to a higher rate of pancreas contamination for longer procurement durations, but these differences were not statistically significant. The duration of organ transport, or cold ischemia time, may present another important variable on pancreas grafts considering that the duodenum is included en bloc with the organ [23]. A significantly increased rate of contamination was observed for cold ischemia times above 4 h. These results are in contrast to reports of contamination rates of kidney transport media, where no correlation was found between contamination rate and cold ischemia time [18]. However, kidney grafts do not have any intestinal structure included and have therefore a lower bio-burden than a pancreas graft that includes the duodenum. The attached duodenum is likely to be the source of contam ination during pancreas preservation and bacterial translocation or bacterial growth may occur during cold ischemia time.

Another possible factor that might influence the rate of pancreas transport media contamination, reported in the literature, might be the different attitudes towards addition of antibiotics to the transport media or different bacteriological sampling or culture techniques used among investigators [22,23,27].

Among the organisms cultured in the pancreas transport medium of our study, the vast majority were Gram-positive organisms, which is in accordance with previous reports [22]. The most frequent germs cultured were staphylococcal species, followed by streptococcal species and *Escherichia coli*, as reported by others [21]. These germs can be found either in the digestive tract or as part of the skin flora.

Previous studies on microbial contamination during islet isolation suggested that a majority of microorganisms were washed, diluted or eliminated during pancreas processing [21–23]. Our results confirm these observations, which showed that the rate of contamination decreased from 56% upon pancreas arrival to 9% after islet preparation purification in Group I, and from 84% to 4% in Group II. As for the transport medium, the majority of infected postpurification preparations showed Gram-positive organisms. The most frequent germs cultured were staphyloccocal species, followed by *Acinetobacter* species and *Candida albicans*. Postpurification preparation contamination could be attributable to the nonclearance of microorganisms during islet isolation or to *de novo* contamination of the preparation during processing. Our results indicated that *de novo* contamination during islet isolation occurred in 5% of noncontaminated pancreata. These contaminations could be attributed to either violation of standard operational procedure during islet isolation process or accidental use of contaminated solutions [22,23].

Microbial contamination during pancreas procurement represents an important bio-burden for the islet isolation process and a source of infection of final islet preparations. While the donor maintenance and the procurement process may be difficult to modify, pancreas decontamination with antiseptic, antibiotic and antimycotic solutions is simple and can further decrease the contamination rate, as it has been shown for corneas [28]. Our results support this view, as the rate of contamination of the final islet preparations in Group II was lower, without reaching statistical significance threshold, than in Group I, despite a higher initial contamination rate of the donor pancreas.

Microbial surveillance of islet isolation process is a reliable approach to trace contaminated islet preparation and to prevent clinically relevant infectious complications in recipients of cellular graft. The importance of microbial surveillance should be emphasized for future clinical trials of islet transplantation, as donor organ contamination during procurement is frequent and *de novo* contamination during islet isolation is not negligible. With the routine use of microbial testing during isolation, we were able to trace all contaminated islet preparations. Moreover, gram testing before transplantation enabled us to disregard all contaminated preparations and resulted in complete absence of microbial pathogen transmission to islet recipients.

In conclusion, the rate of microbial contamination of donor pancreata is high during organ procurement, but most contaminants can be eliminated during islet isolation.

Decontamination of the donor pancreas before the islet isolation process can further reduce microbiological contamination of the final islet preparation.

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References

1. Shapiro AM, Lakey JR, Ryan EA, *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230.
2. Ryan EA, Lakey JR, Rajotte RV, *et al.* Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 2001; **50**: 710.
3. Ryan EA, Lakey JR, Paty BW, *et al.* Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 2002; **51**: 2148.
4. Taylor GD, Kirkland T, Lakey J, Rajotte R, Warnock GL. Bacteremia due to transplantation of contaminated cryopreserved pancreatic islets. *Cell Transplant* 1994; **3**: 103.
5. Isenberg HD, Baron EJF, Dar C Jr, Murray PR, Rodgers FG, A. Recommendations for the isolation of bacteria from clinical specimens. In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ (eds). *Manual of Clinical Microbiology*. Washington, DC: American Society for Microbiology, 1991: 216–221.
6. Benhamou PY, Oberholzer J, Toso C, *et al.* Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000). Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. *Diabetologia* 2001; **44**: 859.
7. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* 1988; **37**: 413.
8. Oberholzer J, Triponez F, Mage R, *et al.* Human islet transplantation: lessons from 13 autologous and 13 allogeneic transplantations. *Transplantation* 2000; **69**: 1115.
9. Hering BJ, Ricordi C. Islet transplantation for patients with type 1 diabetes. *Graft* 1999; **2**: 12.
10. Oberholzer J, Toso C, Ris F, *et al.* Beta cell replacement for the treatment of diabetes. *Ann N Y Acad Sci* 2001; **944**: 373.
11. Pipeleers D, Keymeulen B, Chatenoud L, *et al.* A view on beta cell transplantation in diabetes. *Ann N Y Acad Sci* 2002; **958**: 69.
12. Brayman KL, Stephanian E, Matas AJ, *et al.* Analysis of infectious complications occurring after solid-organ transplantation. *Arch Surg* 1992; **127**: 38.
13. Majeski JA, Alexander JW, First MR, Munda R, Fidler JP, Craycraft TK. Transplantation of microbially contaminated cadaver kidneys. *Arch Surg* 1982; **117**: 221.
14. Spees EK, Light JA, Oakes DD, Reinmuth B. Experiences with cadaver renal allograft contamination before transplantation. *Br J Surg* 1982; **69**: 482.
15. Rowinski W, Pacholczyk M, Chmura A, *et al.* Influence of positive cultures in donor and preservation medium on development of infection in cadaveric kidney transplant recipients: beneficial effects of antibiotic coverage at the time of nephrectomy. *Transplant Proc* 1991; **23**: 2656.
16. Meyers BR, Mendelson MH, Lansman S. Microbial contamination of solid-organ donor transport fluids leading to systemic infection. *Transplantation* 1992; **53**: 1383.
17. Anderson CB, Haid SD, Hruska KA, Etheredge EA. Significance of microbial contamination of stored cadaver kidneys. *Arch Surg* 1978; **113**: 269.
18. Benoit G, Tiguert R, Bensadoun H, *et al.* Incidence of transport medium contamination in cadaver kidney procurement. *Transplant Proc* 1988; **20**: 895.
19. Mora M, Wilms H, Kirste G. Significance of bacterial contamination of cadaver donor renal allografts before transplantation. *Transplant Proc* 1991; **23**: 2648.
20. Pacholczyk MJ, Lagiewska B, Meszaros J, Szostek M, Rowinski W. Bacterial infections transmitted from the donor: antibiotic prophylaxis in the donor. *Transplant Proc* 1996; **28**: 184.
21. Carroll PB, Ricordi C, Fontes P, *et al.* Microbiologic surveillance as part of human islet transplantation: results of the first 26 patients. *Transplant Proc* 1992; **24**: 2798.
22. Scharp DW, Lacy PE, McLear M, Longwith J, Olack B. The bioburden of 590 consecutive human pancreata for islet transplant research. *Transplant Proc* 1992; **24**: 974.
23. Lakey JR, Rajotte RV, Warnock GL. Microbial surveillance of human islet isolation, in vitro culture, and cryopreservation. *Clin Invest Med* 1995; **18**: 168.
24. Bonten MJ, Gaillard CA, Johanson WG Jr, *et al.* Colonization in patients receiving and not receiving topical antimicrobial prophylaxis. *Am J Respir Crit Care Med* 1994; **150**: 1332.
25. Thorens J, Froehlich F, Schwizer W, *et al.* Bacterial overgrowth during treatment with omeprazole compared with cimetidine: a prospective randomised double blind study. *Gut* 1996; **39**: 54.
26. Deutsche Stiftung für Organtransplantation. 2001; http://www.dso.de/ [accessed January 2005].
27. Wood RP, Melendez T, Langnas AN, Navakal H, Kelly D. The viability of microorganisms in preservation solutions. *Transplantation* 1991; **51**: 239.
28. Pels E, Vrensen GF. Microbial decontamination of human donor eyes with povidone-iodine: penetration, toxicity, and effectiveness. *Br J Ophthalmol* 1999; **83**: 1019.