Pancreas preservation fluid microbial contamination is associated with poor islet isolation outcomes - a multi centre cohort study

MEIER, Raphaël, et al.

Abstract
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Pancreas preservation fluid microbial contamination is associated with poor islet isolation outcomes - a multi centre cohort study

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Keywords
Islet isolation, microbial contamination, Islet transplantation, Type 1 diabetes mellitus

Word count
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Author involvement
RM and TB designed the study. RM, PS, DA, CvD, BB, YM, SB, NP, PYB, AW, NN, DB and TB collected the data. RM analyzed the data. RM performed statistical analysis. RM, DA, PS, CvD and TP interpreted the data and wrote the manuscript. RM and TB had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure
The authors have no conflict of interest to disclose.

Abbreviations
Islet equivalents (IEQ), standard deviation (SD), body mass index (BMI), Hank’s balanced salt solution (HBSS)

ABSTRACT
Background
The microbiological safety of islet preparations is paramount. Preservation medium contamination is frequent and its impact on islet yield and function remains unclear.

Methods
Microbiological samples collected during islet isolations from 2006 to 2016 were analyzed and correlated to isolation and allo- and auto-transplantation outcomes.
Results

Microbial contamination of preservation medium was found in 64.4% of processed donor pancreases (291/452). We identified 464 microorganisms including Staphylococcus (253/464, 54.5%), Streptococcus (31/464, 6.7%) and Candida species (25/464, 5.4%). Microbial contamination was associated with longer warm and cold ischemia times and lower numbers of postpurification islet equivalents, purity, transplant rate and stimulation index (all p<0.05). Six percent of the preparations accepted for transplantation showed microbial contamination after isolation (12/200); 9/12 were Candida species. Six patients were transplanted with a sample with late microbial growth discovered after the infusion. Insulin-independence rate was not affected. This risk of transplanting a contaminated islets preparation was reduced by half following the implementation of an additional sampling after 24h of islet culture.

Conclusion

Pancreas preservation fluid microbial contamination is associated with lower transplant rate and poorer in vitro function, but not with changes in graft survival. Culture medium testing one day after isolation reduces the risk of incidental transplantation with contaminated islets.

Introduction

Since the first successful clinical trial of islet transplantation for diabetic patients under the Edmonton protocol [1], the proportion of transplanted recipients with insulin independence has been increasing over the years; from 27% at 3-year (1999–2002) to 44% (2007–2010). Currently up to 50% of well selected patients remain...
insulin independent at five years, almost comparable to pancreas transplant recipients [2-4]. From pancreas procurement, through islet isolation and purification, to islet infusion, the microbiological safety of a preparation intended to be transplanted to an immunosuppressed recipient is of paramount importance [5]. Therefore, a strict quality control process must be implemented. The existing literature describes variable rates of solid organ (kidney, liver, heart, lung, cornea) transport media microbial contamination, ranging from 9% to 64% (median 41%) [6-11]. In comparison, pancreas preservation medium contamination rates were reported between 28% to 62% (median 31%) [12-15]. We previously reported on the microbial surveillance of 215 islet isolations between 1996 and 2002 and showed that pancreas decontamination reduces the risk of microbial contamination of the final islet preparation [14]. At our institution, we enforced a check point at each step from organ procurement, isolation to islet infusion, to ensure clinical safety of the islet transplantation. This study presents the microbial analysis of islet isolation/transplantation, the impact on isolation yield and transplantation outcome, and the implementation of additional safety samplings spanning over 10 years at the University of Geneva.

Methods

Donors

Donor pancreases were procured by operating teams in Swiss University Hospitals, as well as other hospitals within the GRAGIL network [16]. All donor pancreases procured for pancreatic islet isolation and transplantation, a total of 452 from
November 2006 to July 2016, were included in the study. 450 pancreases were retrieved from brain-dead multiorgan donors and two pancreases were retrieved from donors after cardiac death (both Maastricht III). Microbiological samples were prospectively collected for aerobic, anaerobic and fungal microbial cultures as part of a microbiological surveillance protocol to maintain the clinical safety of the islet isolation and transplantation (n=377). Preparations without microbiological sampling or with interrupted procedure before digestion were excluded from the analysis (n=75). The study was approved by the cantonal research ethics committee (protocol no. 2017-00605).

**Pancreas procurement**

Pancreas was procured *en bloc* with the spleen and the duodenum which was routinely flushed though the artery, and placed in preservation medium according to local center preference before being transported to the islet isolation center. Warm ischemia time was defined as the time from cross-clamp to pancreas cut-out. The preservation media included: University of Wisconsin solution (UW), Institut Georges Lopez-1 solution (IGL-1), Celsior, Scot, Histidine-tryptophan-ketoglutarate (HTK) and other media. The duodenum was left attached to the pancreas and stapled without prior decontamination.

**Islet isolation and microbiological sampling**

Samples of 10ml (thereafter microbiological samples) were collected from each step for microbiological cultures: after donor procurement, after purification and before islet transplantation. The first microbiological sample was taken from the donor.
pancreas preservation medium upon its arrival and unpacking from the donor organ preservation bag (Sample A). A decontamination protocol of the pancreas was undertaken as previously described [14]. Briefly, after removal of the spleen and trimming of the pancreas from the vessels and adipose tissue, the pancreatic duct was clamped and the pancreas was placed successively for 30 seconds in a 5% polyvidon-iodine bath (Betadine®, Mundipharma, Basel, Switzerland), a cefazoline/amphotericine B bath (1 g cefazoline (Kefzol®, Lilly, Vernier, Switzerland) and 100 mg amphotericine B (Fungizon®, Bristol-Myers Squibb, Baar, Switzerland) in 150 ml of Hank’s balanced salt solution (HBSS)). Then, the pancreas was rinsed in three successive cold HBSS solutions. Islets were isolated using the automated method described by Ricordi et al. with local modifications [17-22] using either Liberase HI® (Roche, Indianapolis, IN), Collagenase NB1 or Collagenase NB2® (Serva Electrophoresis, Heidelberg, Germany). Islets were purified on a continuous Biocoll® gradient (Biochrom, Berlin, Germany) with a refrigerated COBE cell processor (COBE 2991®; Cobe, Lakewood, CO). The second microbiological sample was collected from the supernatant solution immediately after islet purification and washing with CMRL medium containing penicillin (50 U/ml) and streptomycin (50 µg/ml) (Roche GmbH, Mannheim, Germany) (Sample B, wash medium). This represented a safety check point before the islets were cultured. In 2012, we implemented an additional microbiological sampling of the culture media after 24 hours of culture (sample B1, culture medium). Once the islets were deemed suitable for transplantation, a third microbiological sample was collected for both culture (Sample C, transplantation medium) and direct examination (Gram staining) from the solution used to wash the final islets preparation immediately before packing into the infusion bag. All microbiological samples (A, B, B1 and C) were injected (10 ml).
under aseptic technique into blood culture flasks (BACTEC Plus aerobic/F, BACTEC Lytic/10 Anaerobic/F and BACTEC Myco/F-lytic, Becton Dickinson, Sparks, MD, USA) and dispatched to our Bacteriology Laboratory [14]. Sample A (preservation medium) was collected under a laminar flow (class B (2006-2008) and A (2009-2016) environment). Samples B, B1, and C (including the Gram staining) were collected under a class A safety cabinet. A flowchart indicating the timing when microbiological samples A, B, B1, C were collected is shown in Figure 1. A negative Gram staining result of the final sample (sample C) was mandatory before islet transplantation. Microbiological samples were cultured using standard procedures; at least 5 days at 35°C. If positive, the microbial organisms were characterized based on the recommendations provided in the Manual of Clinical Microbiology [23]. Low and high virulence microorganisms were defined as species known to cause significant infections in immunocompromised patients [10]. All islet recipients received standard intravenous antibioprophylaxis (Cefazolin, 1g) 20 minutes prior to islet injection.

**Islet quantity and quality assessment**

Islet counting and purity assessments were performed before and after purification as previously described [24]. The number of islet equivalents (IEQ) was calculated by normalizing the islets to a standard diameter of 150 µm [25]. Transplant volume, viability, purity, endotoxin levels and functionality of islet preparations were assessed just before transplantation (n=189). Islet viability was assessed by fluorescein diacetate and propidium iodide staining as previously described [26]. Endotoxin levels were measured using the Endosafe-Portable Test System (Charles River...
Laboratories, Wilmington, MA). *In vitro* function was assessed one week after transplantation using a static glucose-stimulated insulin release assay. The stimulation index was calculated as the ratio of insulin concentration of stimulated (high glucose, 16.7 mM) to basal (low glucose, 2.8 mM) conditions. Islet preparation were deemed suitable for transplantation per the following releasing criteria: ≥ 4’000 IEQ/kg of recipient’s body weight, final purity of the preparation ≥ 30%, final viability of the preparation ≥ 70%, total volume of the preparation ≤ 10 ml, endotoxin level ≤ 5 EU/kg of recipient's body weight, negative Gram staining.

**Recipients**

A total of 189 islet preparations were transplanted to 92 recipients. Transplanted patients were divided into two groups depending on the presence or absence of microbiological contamination of the preservation solution. Preparation without microbiological testing of the preservation medium where excluded from analysis (n=3). Transplants were performed in the GRAGIL network [24] as allotransplantation (n=176, simultaneous islet kidney, islet after kidney, islet transplant alone or islet after lung) or autotransplantation (n=13) in different previously reported protocols [4, 27-30]. All recipients received between one and three islet preparations intraportally through a percutaneous transhepatic approach. Immunosuppression consisted in steroid-free regimens modified from the original "Edmonton protocol" associating anti-CD25 mAb, anti-thymocyte globulin and TNF inhibitor (induction), as well as FK506 and mycophenolic acid (maintenance) [31]. Islet graft survival and function were assessed at 1, 6, 12, 24, 36 and 48 months after the first islet injection. Patients were excluded at these time points if they had
received another islet injection with a different contamination status as compared to their initial contamination status. Patient with an auto-transplantation were excluded from survival analysis.

**Statistical analysis**

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequencies (%). Differences between groups were analyzed with the Student t-test of Mann-Whitney U test for continuous variables and the Chi-square test for binary and categorical variables, and multivariate logistic regression. Survival analyses were performed with the Kaplan–Meier method and the Gehan-Breslow-Wilcoxon test. An exact two-sided p value <0.05 was considered statistically significant.

**Results**

**Microbial analysis before islet isolation**

Over a ten-year period (November 2006 to July 2016), 452 donor pancreases were processed at our islet isolation center. Cultures of preservation fluids following pancreas procurement and transportation (sample A) were performed in 377 of these pancreases. Contamination by microorganisms, proven by positive cultures, occurred in 291 (64.4%) preservation media (Table 1). More than one germ grew in 41.2% of the positive samples (120/291) (Figure 2). The majority of the 464 identified microorganisms were bacteria (438; 94.4%) and the remainders were fungi (26; 5.6%) (Figure 3; Supplementary Table 1). Overall, Gram-positive bacteria
predominated (355/464, 76.5%). *Staphylococcus* was the most frequently encountered genus (253/464, 54.5%), with a majority of coagulase negative staphylococci (209/464, 45.0%) (Figure 3). The second most frequently identified bacterial genus was *Streptococcus* (31/464, 6.7%). *Enterobacteriaceae* (*Escherichia coli, Klebsiella pneumoniae, Enterobacter*) were the most frequent Gram-negative bacteria (43/464, 9.3%). Notably, no significant acquired antibiotic resistance was identified (particularly neither methicillin-resistant *Staphylococcus aureus* (MRSA) nor Extended-spectrum beta-lactamases bacteria (ESBL)). Of the 26 fungi identified (26/464, 5.6%), half were *Candida albicans*.

To identify risk factors for contamination we compared demographic variables of donors corresponding to the 291 contaminated samples to the 86 donors with sterile samples (Table 2). Longer warm (p<0.001) and cold ischemia times (p=0.046) were associated with contaminated preservation medium (Table 2) and higher numbers of microbial species (Figure 4A, B). The average numbers of microbial species found in the different types of preservation media are shown in Figure 5.

Outcomes of the islet isolation procedure were then compared between the two groups (initial contamination vs. no contamination) (Table 3). Pancreas weight, digested tissue weight and tissue volume were higher in the contaminated group. The number of islet equivalents (IEQ) before purification was similar between pancreases with and without preservation fluid contamination: 342’404 ± 185’906 IEQ and 345’774 ± 191’021 IEQ, respectively, p=0.883. Consistent with an increased pancreas weight, a lower IEQ per gram of pancreas was observed in the contaminated group (p=0.036). More fragmented islets were observed in the contaminated group. Following purification, contaminated media were associated
with significantly lower islet yield (245'597 ± 140'619 IEQ vs. 287'495 ± 168'973 IEQ, p=0.028) and had a lower IEQ per gram of pancreas following purification (p=0.004). Mean islet size was smaller in the contaminated group after purification (155.6 ± 61.5 vs. 178.4 ± 98.9, p=0.014). Islet preparations were less likely to be transplanted in the contaminated group (45.7% vs. 65.1%, p<0.001). Considering transplantation as an independent variable and age, sex, BMI, pancreas weight, ICU stay, cause of death, warm and cold ischemia, and preservation fluid contamination status as independent variables, a multivariate regression identified low BMI (p=0.002), UW use (p=0.005) and preservation fluid contamination (p=0.043) as independent factors associated with failure to proceed towards transplantation.

Preservation fluid contamination also affected quality control parameters assessed following isolation in preparations released for transplantation (Table 4). Preparations from pancreases with contaminated preservation fluids had significantly lower stimulation indexes and higher endotoxin contents. Moreover, they had lower purity, and thus higher transplant volume. Four-year insulin independence rate and graft function were not different between both groups (Figure 6).

**Microbial analysis during and after islet isolation**

After islet isolation and purification, 44.2% (200/452) of islet preparations met the release criteria for transplantation. Out of these, 70% (140/200) had a culture positive sample A (preservation medium). Only one sample B was culture positive (*Staphylococcus epidermidis*) (1/200, 0.5%), the corresponding sample A (preservation medium) being culture positive for the same bacterium. The absence of bacteria in further bacteriological samplings highlights the efficacy of the...
successive washing steps during digestion, isolation and purification in eliminating microorganisms inherited from donor pancreas procurement. Of note, this single sample B culture positive result came back after transplantation and the recipient did not develop signs of infection (a standard antibiotic prophylaxis, which did not cover *Staphylococcus epidermidis*, was given to the recipient).

**Microbial analysis before islet transplantation**

After islet isolation and culture, microbiological samples were collected from the transplantation media (sample C). Microorganisms grew from 12 samples C (6.0%). For four culture positive samples C, no corresponding sample B had been performed. Of note, eight culture positive samples came from islets with a sterile sample B, suggesting either growth of microorganisms not eliminated by the isolation process (and present in undetectable numbers in sample B), or microbial contamination during the islet culture itself. The culture results of sample C (transplantation media) were accessible only several days after transplantation. Importantly, 75% (9/12) of culture positive samples C grew *Candida* species. In the 12-corresponding sample A (preservation medium) that were followed by a culture positive sample C (transplantation media), 42% (5/12) were initially culture positive for *Candida* species.

After collecting sample C (i.e. immediately before transplantation), a Gram-staining was performed and detected seven contaminated preparations, all identified thereafter by culture positive samples C (Table 5). Six of these preparations were discarded and not transplanted (Table 6), and one preparation was transplanted while the positive culture result was obtained retrospectively (i.e. >30 min after the
preparation packing) (Table 7). Overall, 96.0% of the preparations released for transplantation were finally transplanted (192/200), accounting for 42.5% of the islet isolation procedures (192/452). Of note, two islet preparations were discarded for non-microbiological reasons. A total of six patients were transplanted with a contaminated preparation (positive sample C, transplantation media); among those, five had a prior negative Gram-staining. All six were treated preemptively with antibiotics or antifungals (Table 7). Patient F had a candida esophagitis that was successfully treated with 10 days of fluconazole; postoperatively, the patient suffered from polyarthritis of unknown origin. The other patients remained asymptomatic. The patients transplanted with a contaminated islet preparation had similar four-year insulin independence rate compared to the other transplanted patients (p=0.853).

In 2012, we implemented an additional microbiological sampling after 24 hours of culture (sample B1). Over the period 2012 to 2016 this sample was culture positive on four occasions, and allowed a 50% reduction in the number of preparations transplanted with a sample C (transplantation media) being positive afterwards. During the period 2006 to 2011 four patients were transplanted with culture positive samples C preparations versus two during the period 2012 to 2016 (Figure 7).

Discussion

In this study, we report the results of microbiological analysis of pancreas preservation media and the potential risk factors for microbial contamination in the process of clinical islet isolation. Data were prospectively collected as part of our microbiological surveillance protocol, performed to safeguard islet transplantation at our center and in the GRAGIL network. We observed that longer cold and warm...
ischemia times were associated with a higher risk of microbial contamination, and that pancreases with contaminated preservation fluids less likely meet release criteria for transplantation. In 2012, we implemented an additional microbiological sampling that reduced by half the contamination rates of the final product, although the number of contaminated preparations was too low to draw compelling conclusions.

Our microbiological analysis of 452 donor pancreases spanning the last 10 years confirmed a high incidence (64.4%) of microbiological contamination, compatible with previous published rates varying between 19% and 68% [12-15, 32]. Differences between published contamination rates may be explained by inconsistent use of iodine duodenum decontamination, proton-pump inhibitors in the intensive care unit, and other variations in the retrieval and microorganism identification protocols. As a point of comparison, the rate of microbial contamination of the preservation medium for other organs are as follows: 9% to 57% for kidneys [6, 8], in 27% to 62% for livers [7, 8], in 45% to 64% for heart valves [10], in 29% for lungs [9] and in 14% to 29% for cornea [11, 33]. In the present study, we identified a majority of Gram-positive bacteria namely *Staphylococcus* spp. and *Streptococcus* spp.; which is in accordance with our previously published results (period 1996 - 2002) [14]. It is possible that a proportion of *Staphylococcus*-positive samples were contaminated by skin flora during the process of pancreas procurement and/or placement in preservation medium. Most other microorganisms identified are part of the natural flora of the digestive tract. The procurement of a pancreas includes a section of the duodenum, potentially source of such microbiological contamination. Consistent with a donor community-acquired profile, and short ICU stays (2.4 days) restricting potential exposure to antibiotics, we identified no multi-resistant bacteria. In
response to the high contamination rates of pancreas preservation fluids, these microorganisms were successfully eliminated by a decontamination protocol and subsequent washing steps during islet isolation. This allowed decreasing contamination rates from 64.4% to 0.5% immediately after isolation and 6.0% after culture.

The number of microorganisms found after donor pancreas procurement was a small but with significant proportion of fungi (5.6%). Mostly Candida species were present among the germs detected after the isolation process (75%). This may be the result of a selection due to the presence of penicillin and streptomycin and the absence of antifungal in the culture media. A target therapy may be considered, however our preliminary experimental results using amphotericin B in the culture medium of human islet showed a narrow therapeutic window with a decrease in islet viability (data not shown). The main mechanism of final preparation contamination was the persistence of germs present in the culture medium after retrieval. Possible de novo microbial contamination concerned 2.5% preparations. This number was consistent with what we previously reported [14]. De novo contamination may be caused by manipulation mistakes or accidental use of contaminated solutions [13, 32], or false negative culture of preservation medium.

The sterile technique during islet purification and culture continues to be of utmost importance. In a ten-year period, there were 6 out of 200 transplanted preparations that were subsequently found to be contaminated. Recipients were treated accordingly and fortunately there was no directly associated infectious complication and insulin-independence rates were similar to those of other recipients. Interestingly, we successfully implemented a routine analysis of the culture medium.
24h after isolation that allowed a further reduction in the number of contaminated transplanted preparations.

The number of microorganisms retrieved in the preservation medium increased with longer warm and cold ischemia times. This represents a further argument in favor of keeping these times as short as possible [34]. Also, this may be of growing interest because of the current ongoing shift from brain-dead donors towards donors after circulatory death, in whom the warm ischemia time is increased and poorly controlled. As the current study concerns mostly pancreas from brain-dead donor, it could be used as a reference for future studies with donors after circulatory death.

Pancreases with contaminated preservation culture medium were associated with lower islet yields, and were less likely to be transplanted. Interestingly, the multivariate model confirmed that the preservation fluid contamination was independently associated with a failure to transplant the preparation; whereas warm and cold ischemia and pancreas weight were not. Consistent with our findings, in an auto-transplantation setting, Jolissaint et al. also found that bacterial contaminants in the final islet preparation were associated with lower islet yield and lower C-peptide/insulin independence rates in 6 patients [35]. However, such small number of patients does not allow definitive conclusion and the situation could be different in this latter study because contamination is usually present at a higher load in patient with chronic pancreatitis and pancreatic duct dilatation and obstruction.

Interestingly, the two groups that we compared (initial contamination vs. no contamination) had similar prepurification IEQ; but the situation changes following purification and there was significantly lower IEQ in the contaminated group, reflecting an inability to recover islets from the exocrine tissue. The increased
pancreas weight in the contaminated group is another argument of poor quality, possibly caused by cellular edema that is associated with longer warm ischemia times and that causes modified islet density and thus difficult Biocoll gradient purification. Most surprisingly, the in vitro islet function reflected by stimulation index was lower in the contaminated group. This indicates a poorer islet quality in the latter group. Nevertheless, islet survival and function following transplantation were not affected. The absence of difference at this level could be explained by the fact that the “poorest” contaminated preparations were not transplanted because of insufficient IEQ numbers. The detrimental effect of microbial contamination on islet function may also be transient and thus reflected in vitro but not in vivo. Finally, the in vivo situation differs from the in vivo one by the fact that antibiotic prophylaxis is given and some anti-microbial immunity could be expected in the transplanted patients.

Microbial analysis at different steps of the donor pancreas procurement, islet isolation and transplantation is a mandatory approach to safeguard the safety of islet transplantation, and to prevent iatrogenic infectious complications in immunosuppressed patients. Pancreas preservation medium contamination seems to directly negatively impact on islet yield and quality. Accordingly, methods to reduce initial contamination should be used more routinely or investigated, such as the removal of duodenal segment from pancreas prior to packaging, iodine decontamination of the duodenum, or antifungal or antibiotics supplementation of the preservation medium. Evidence provided by these results strengthen the fact that warm and cold ischemia times should be kept as short as possible. Furthermore, based on our results, a microbiological testing of culture medium after 24h should be performed as it further reduces the risk of contamination of the transplant product.
References


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Legends to figures

**Figure 1:** Flowchart indicating the timing when culture samples A, B, B1, C and Gram stain samples are collected.

**Figure 2:** Histogram showing the number of islet isolation(s) with 1, 2, 3, 4, 5 or 6 microorganism species found in the pancreas preservation medium.

**Figure 3:** Most frequently found microorganisms in the pancreas preservation media. Percentage are relative to the total number of germs identified. High- and low-risk microorganisms are shown.

**Figure 4:** Graph showing number of microorganism(s) found in the pancreas preservation medium as a function of the (A) warm and cold (B) ischemia times.

**Figure 5:** Number of microorganism(s) found in the pancreas preservation medium classified in function of the type of preservation solution used.

**Figure 6:** Survival curves for (A) insulin Independence and (B) C-peptide positivity in preparation with and without preservation medium contamination (both p values > 0.05). Gehan-Breslow-Wilcoxon test was used for survival curves comparison.

**Figure 7:** Number of preparations with culture positive sample C that were processed in the view of transplantation during period 2006-2011 (no bacteriological sampling after 24h of islet culture) and period 2012-2016 (bacteriological sampling after 24h of islet culture: sample B1). Light gray: not transplanted; black: transplanted. In the 2012-2016 period, one of the transplanted patient had a culture
positive sample B1 result after transplantation and one patient had sample B1 not collected.

Legends to tables:

Table 1: contamination rate of pancreas preservation media upon arrival in the isolation center (sample A).

Table 2: demographic variables of donors in the group with microbial contamination versus the group with no contamination of the pancreas preservation medium.

Table 3: Comparison of outcomes of the islet isolation procedure for the group with microbial contamination versus the group with no contamination of the pancreas preservation medium.

Table 4: Quality control variables of transplanted preparations in the group with microbial contamination versus no contamination in the pancreas preservation medium.

Table 5: analysis of bacteriological samples collected from islet preparations accepted for further culture and transplantation throughout the processes of purification and culture. Sample A: preservation medium collected upon pancreas arrival. Sample B: wash medium collected after the purification and immediately before culture. Sample C and Gram staining sample (transplantation medium): collected after islets culture and immediately before transplantation.

Table 6: islet preparations not transplanted due to positive Gram staining.

Table 7: Transplanted islet preparations with positive microbiological analysis found to be positive after transplantation.

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Supplementary table 1: complete list of all microorganisms found in the pancreas preservation media.

Acknowledgments / Funding

The current work was supported by Geneva University Hospital and University of Geneva

Table 1

<table>
<thead>
<tr>
<th>Sample A (preservation medium)</th>
<th>Pancreas isolation (n=452)</th>
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<tbody>
<tr>
<td>- Positive (%)</td>
<td>291 (64.4)</td>
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<tr>
<td>- Negative (%)</td>
<td>86 (19.0)</td>
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<td>- Not done (%)</td>
<td>75 (16.6)</td>
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Table 2

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<th>Variables</th>
<th>Presence of microbial contamination in the preservation medium (n = 291)</th>
<th>Absence of microbial contamination in the preservation medium (n = 86)</th>
<th>p-value *</th>
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<td>Age, yr ± SD (min - max)</td>
<td>47.1 ± 13.5 (6-70)</td>
<td>48.8 ± 13.3 (9-71)</td>
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<tr>
<td>Sex</td>
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<td></td>
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<tr>
<td>Male (%)</td>
<td>174 (59.8)</td>
<td>41 (47.7)</td>
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<td>Female (%)</td>
<td>117 (40.2)</td>
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<table>
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<tr>
<th>Variables</th>
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<th>Absence of microbial contamination in the preservation medium (n = 86)</th>
<th>p-value *</th>
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<tbody>
<tr>
<td>Pancreas weight, g</td>
<td>101.0 ± 24.7</td>
<td>88.3 ± 28.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Undigested tissue weight, g</td>
<td>16.4 ± 12.5</td>
<td>15.1 ± 11.3</td>
<td>0.365</td>
</tr>
</tbody>
</table>

BMI, body mass index; ICU, intensive care unit; UW, University of Wisconsin solution; IGL-1, Institut Georges Lopez-1 solution; IEQ, islet equivalent.

* Student t-test for continuous variables and chi-square test for binary or categorical variables (global P-value).
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<th>Autograft</th>
<th>p-value</th>
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<td>Digested tissue weight, g</td>
<td>84.4 ± 23.4</td>
<td>73.2 ± 25.6</td>
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<td>Digestion rate, %</td>
<td>83.8 ± 11.7</td>
<td>83.1 ± 11.4</td>
<td>0.625</td>
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<td>Digestion time, min</td>
<td>18.3 ± 4.1</td>
<td>18.8 ± 4.3</td>
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<td>Tissue volume, ml</td>
<td>44.5 ± 16.4</td>
<td>37.1 ± 15.7</td>
<td>&lt;0.001</td>
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<td>Total number of islets, prepurification</td>
<td>374'391 ± 174'666</td>
<td>457'989 ± 819'814</td>
<td>0.107</td>
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<tr>
<td>IEQ prepurification</td>
<td>342'404 ± 185'906</td>
<td>345'774 ± 191'021</td>
<td>0.883</td>
</tr>
<tr>
<td>IEQ/per g pancreas (prepurification)</td>
<td>3'529 ± 2'018</td>
<td>4'059 ± 2'175</td>
<td>0.036</td>
</tr>
<tr>
<td>Mean prepurification islet size, µm</td>
<td>147.2 ± 69.3</td>
<td>144.2 ± 66.5</td>
<td>0.717</td>
</tr>
<tr>
<td>Embedded islets, %, prepurification</td>
<td>23.5 ± 20.4</td>
<td>23.8 ± 19.3</td>
<td>0.902</td>
</tr>
<tr>
<td>Fragmented islets, %, prepurification</td>
<td>12.8 ± 8.4</td>
<td>10.4 ± 5.2</td>
<td>0.021</td>
</tr>
<tr>
<td>Total number of islets postpurification</td>
<td>257'984 ± 158'424</td>
<td>289'808 ± 207'432</td>
<td>0.149</td>
</tr>
<tr>
<td>IEQ postpurification</td>
<td>245'597 ± 140'619</td>
<td>287'495 ± 168'973</td>
<td>0.028</td>
</tr>
<tr>
<td>IEQ/per g pancreas (postpurification)</td>
<td>2'560 ± 1'471</td>
<td>3'139 ± 1'851</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean postpurification islet size, µm</td>
<td>155.6 ± 61.5</td>
<td>178.4 ± 98.9</td>
<td>0.014</td>
</tr>
<tr>
<td>Recovery rate, %</td>
<td>79.1 ± 42.7</td>
<td>86.0 ± 50.4</td>
<td>0.234</td>
</tr>
<tr>
<td>Isolation success (i.e., final yield ≥250 000 IEQ) (%)</td>
<td>133 (45.7)</td>
<td>44 (51.2)</td>
<td>0.069</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Allotransplantation</td>
<td>131 (45.0)</td>
<td>45 (52.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- Autotransplantation</td>
<td>2 (0.7)</td>
<td>11 (12.8)</td>
<td></td>
</tr>
<tr>
<td>- Not transplanted</td>
<td>158 (54.3)</td>
<td>30 (34.9)</td>
<td></td>
</tr>
</tbody>
</table>

IEQ, islet equivalent.

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* Student t-test for continuous variables and chi-square test for binary or categorical variables (global P-value).

Table 4

<table>
<thead>
<tr>
<th>Variables</th>
<th>Presence of microbial contamination in the preservation medium (n = 133)</th>
<th>Absence of microbial contamination in the preservation medium (n = 56)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed transplant volume (ml)</td>
<td>2.1 ± 0.9</td>
<td>2.8 ± 2.9</td>
<td>0.031</td>
</tr>
<tr>
<td>Viability, % (FDA/PI)</td>
<td>89.9 ± 4.1</td>
<td>89.8 ± 4.4</td>
<td>0.870</td>
</tr>
<tr>
<td>Purity, %</td>
<td>64.0 ± 16.0</td>
<td>56.7 ± 18.6</td>
<td>0.012</td>
</tr>
<tr>
<td>Stimulation index</td>
<td>1.6 ± 0.8</td>
<td>2.1 ± 2.1</td>
<td>0.021</td>
</tr>
<tr>
<td>Endotoxin contents (EU/ml)</td>
<td>0.51 ± 0.17</td>
<td>0.43 ± 0.09</td>
<td>0.012</td>
</tr>
</tbody>
</table>

* Student t-test for continuous variables.

Table 5

<table>
<thead>
<tr>
<th>Islet preparations accepted for further culture and transplantation (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A (preservation medium)</td>
</tr>
<tr>
<td>- Positive (%)</td>
</tr>
<tr>
<td>- Negative (%)</td>
</tr>
<tr>
<td>- Not done (%)*</td>
</tr>
<tr>
<td>Sample B</td>
</tr>
<tr>
<td>- Positive (%)</td>
</tr>
<tr>
<td>- Negative (%)</td>
</tr>
</tbody>
</table>

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Table 6

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Year</th>
<th>IEQ</th>
<th>Microbial contamination (Sample A, preservation medium)</th>
<th>Microbial contamination (Sample B)</th>
<th>Microbial contamination (Sample C, transplantation media)</th>
<th>Gram staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2007</td>
<td>208'542</td>
<td>Enterococcus sp. Staphylococcus aureus</td>
<td>Negative</td>
<td>Candida albicans</td>
<td>Positive</td>
</tr>
<tr>
<td>B</td>
<td>2011</td>
<td>449'708</td>
<td>Coagulase-negative staphylococci Candida albicans</td>
<td>Negative</td>
<td>Candida albicans</td>
<td>Positive</td>
</tr>
<tr>
<td>C</td>
<td>2012</td>
<td>412'500</td>
<td>E. Coli Enterococcus faecalis Proteus mirabilis Candida lusitaniae</td>
<td>Negative</td>
<td>Candida robusta Candida norvegensis</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Three preparations were transplanted without Sample A being performed and were thus not counted in Table 2, 3 and 4. ** Six preparations were discarded due to positive gram staining and two preparations were discarded for non-microbiological reasons.
<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>Number</th>
<th>Organism(s)</th>
<th>Result 1</th>
<th>Organism(s)</th>
<th>Result 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>2013</td>
<td>238'500</td>
<td>Peptostreptococcus magnus</td>
<td>Negative</td>
<td>Candida robusta</td>
<td>Positive</td>
</tr>
<tr>
<td>E</td>
<td>2013</td>
<td>266'444</td>
<td>Citrobacter freundii, Enterococcus faecalis, Enterococcus faecium, Klebsiella pneumoniae, Staphylococcus aureus, Candida robusta</td>
<td>Negative</td>
<td>Candida robusta</td>
<td>Positive</td>
</tr>
<tr>
<td>F</td>
<td>2015</td>
<td>259'972</td>
<td>Staphylococcus epidermidis</td>
<td>Negative</td>
<td>Candida albicans</td>
<td>Positive</td>
</tr>
<tr>
<td>Patient</td>
<td>Year</td>
<td>Age</td>
<td>Sex</td>
<td>IEQ</td>
<td>Type of graft</td>
<td>Microbial contamination (Sample A, preservation medium)</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>--------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>A</td>
<td>2007</td>
<td>35.0</td>
<td>F</td>
<td>55'667</td>
<td>Autotransplantation</td>
<td><em>Staphylococcus</em> sp.</td>
</tr>
<tr>
<td>B</td>
<td>2008</td>
<td>49.9</td>
<td>M</td>
<td>279'389</td>
<td>Allotransplantation</td>
<td><em>Neisseria</em> sp. <em>Alpha-hemolytic Streptococcus</em> <em>Staphylococcus capitis</em></td>
</tr>
<tr>
<td>C</td>
<td>2010</td>
<td>44.8</td>
<td>M</td>
<td>289'083</td>
<td>Allotransplantation</td>
<td>ND</td>
</tr>
<tr>
<td>D</td>
<td>2011</td>
<td>56.7</td>
<td>F</td>
<td>262'542</td>
<td>Allotransplantation</td>
<td><em>Candida albicans</em> <em>Streptococcus parasanguinis</em></td>
</tr>
<tr>
<td>E</td>
<td>2012</td>
<td>51.0</td>
<td>F</td>
<td>491'167</td>
<td>Allotransplantation</td>
<td><em>Staphylococcus aureus</em> <em>alpha-hemolytic Streptococcus</em> <em>Candida albicans</em></td>
</tr>
<tr>
<td>F</td>
<td>2013</td>
<td>63.1</td>
<td>F</td>
<td>320'667</td>
<td>Allotransplantation</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Transplanted preparation without the information on Gram staining

ND: not done

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Figure 1

Preservation fluid → Sample A

Pancreas digestion

Washing steps

Tissue purification

Washing steps → Sample B

Islet culture → Sample B1

Islet transplantation → Sample C + Gram staining
Figure 2

![Bar chart showing the number of isolations for different numbers of microorganism species.](chart.png)
Figure 3

![Bar chart showing the number of times various microorganisms were identified as high virulence or low virulence.](chart.png)

- **High virulence microorganisms**
  - *Coagulase-negative staphylococcus*: 200
  - *Staphylococcus aureus*: 44
  - *Propionibacterium*: 31
  - *Candida spp.*: 25
  - *Lactobacillus spp.*: 19
  - *Escherichia coli*: 16
  - *Klebsiella spp.*: 15
  - *Enterococcus spp.*: 14
  - *Haemophilus influenzae*: 9
  - *Pseudomonas spp.*: 7
  - *Hafnia alvei*: 6

- **Low virulence microorganisms**
  - *Enterobacter spp.*: 12
  - *Serratia spp.*: 11

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Figure 4

A

B

Cold ischemia (min)  

Warm ischemia (min)  

Number of microbial species

0 - 100 min  

100 - 200 min  

200 - 300 min  

300 - 400 min  

400 - 500 min  

500 - 600 min  

> 600

0 - 20 min  

20 - 40 min  

40 - 60 min  

60 - 80 min  

80 - 100 min  

100 - 120 min  

> 120

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Figure 6

(A) Contaminated vs. Non-contaminated

(B) Contaminated vs. Non-contaminated

p > 0.05
Figure 7

Number of preparation with positive sample C, candidate for transplantation

- 2006 - 2011
  - Not transplanted
  - Transplanted

- 2012 - 2016
  - Not transplanted
  - Transplanted