ORIGINAL ARTICLE

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

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SUMMARY

The microbiological safety of islet preparations is paramount. Preservation medium contamination is frequent, and its impact on islet yield and function remains unclear. Microbiological samples collected during islet isolations from 2006 to 2016 were analyzed and correlated to isolation and allo- and autotransplantation outcomes. 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 of 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 24 h of islet culture. 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 1 day after isolation reduces the risk of incidental transplantation with contaminated islets.

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Key words
islet isolation, islet transplantation, microbial contamination, type 1 diabetes mellitus

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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 5 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, and 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% and 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. A total of 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 10 ml (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% polyvidonum-iodine bath (Betadine®; Mundipharma, Basel, Switzerland), a cefazolin/amphotericin B bath [1 g cefazoline (Kefzol®; Lilly, Vernier, Switzerland), and 100 mg amphotericin 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.* [17–22] with local modifications using either Liberase HI® (Roche, Indianapolis, IN, USA), 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, USA). The second microbiological sample was collected from the supernatant solution immediately after islet purification and

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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 h 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 class 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, and C were collected is shown in Fig. 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, 1 g) 20 min prior to islet injection.

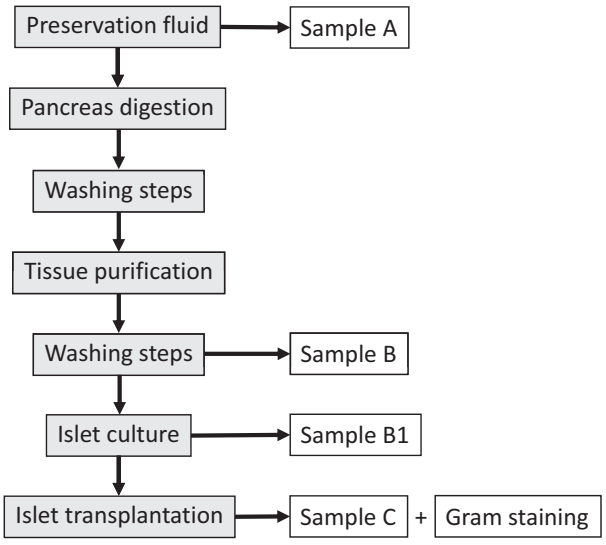

Figure 1 Flowchart indicating the timing when culture samples A, B, B1, C, and Gram stain samples are collected.

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, USA). *In vitro* function was assessed 1 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 was deemed suitable for transplantation per the following releasing criteria: ≥4000 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 was 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) [1]. 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 autotransplantation was 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 10-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) (Fig. 2). The majority of the 464 identified microorganisms were bacteria (438; 94.4%) and the remainders were fungi (26; 5.6%) (Fig. 3; Table S1). 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%) (Fig. 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 (Fig. 4a,b). The average numbers of microbial species found in the different types of preservation media are shown in Fig. 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

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

	Pancreas isolation (n = 452)
Sample A (preservation medium)	
Positive (%)	291 (64.4)
Negative (%)	86 (19.0)
Not done (%)	75 (16.6)

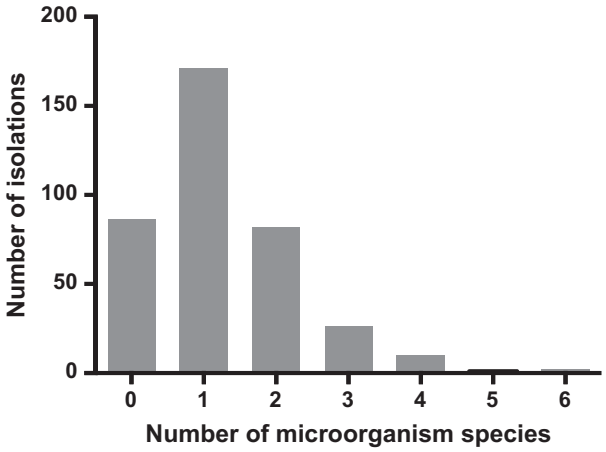

Figure 2 Histogram showing the number of islet isolation(s) with 1, 2, 3, 4, 5, or 6 microorganism species found in the pancreas preservation medium.

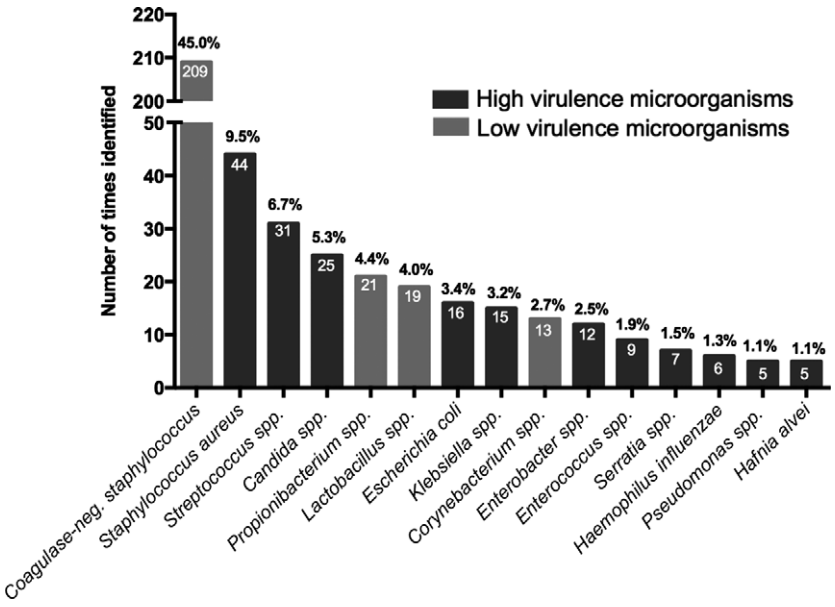

Figure 3 Most frequently found microorganisms in the pancreas preservation media. Percentage are relative to the total number of germs identified. High- and low-risk microorganisms are shown.

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

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

Variables	Presence of microbial contamination in the preservation medium ($n = 291$)	Absence of microbial contamination in the preservation medium ($n = 86$)	*P*-value*
Age, year ± SD (min–max)	47.1 ± 13.5 (6–70)	48.8 ± 13.3 (9–71)	0.309
Sex			
Male (%)	174 (59.8)	41 (47.7)	0.048
Female (%)	117 (40.2)	45 (52.3)	
BMI, kg/m^2 ± SD	25.7 ± 6.1	25.1 ± 6.4	0.417
ICU stay, days ± SD	2.5 ± 1.8	2.5 ± 2.4	0.741
Cause of death			
Cerebral trauma (%)	181 (62.2)	53 (61.6)	0.079
Cerebrovascular (%)	68 (23.4)	22 (25.6)	
Suicide (%)	21 (7.2)	2 (2.3)	
Anoxia (%)	13 (4.5)	2 (2.3)	
Others	8 (2.7)	7 (8.1)	
Preservation solution			
UW (%)	47 (16.2)	13 (15.1)	0.171
IGL-1 (%)	146 (50.2)	47 (54.7)	
Celsior (%)	61 (21.0)	10 (11.6)	
Other (%)	37 (12.7)	16 (18.6)	
Warm ischemia time, min ± SD	67.5 ± 28.0	54.9 ± 25.6	<0.001
Cold ischemia time, min ± SD	380.7 ± 157.4	340.4 ± 176.3	0.046

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).

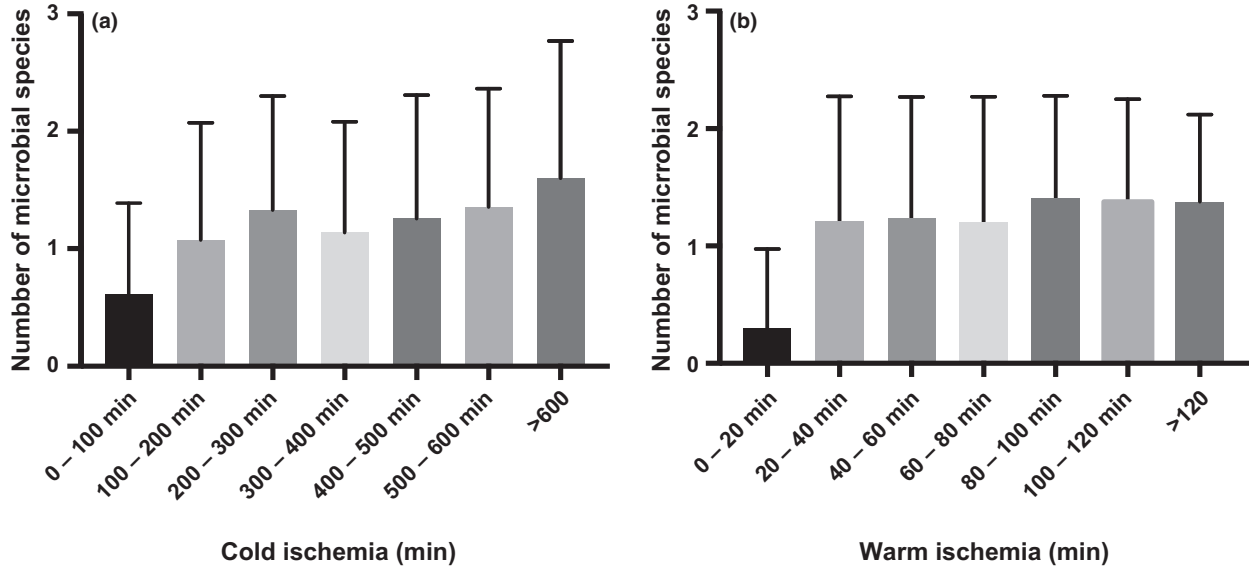

Figure 4 Graph showing number of microorganism(s) found in the pancreas preservation medium as a function of the (a) warm and cold (b) ischemia times.

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 toward 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 (Fig. 6).

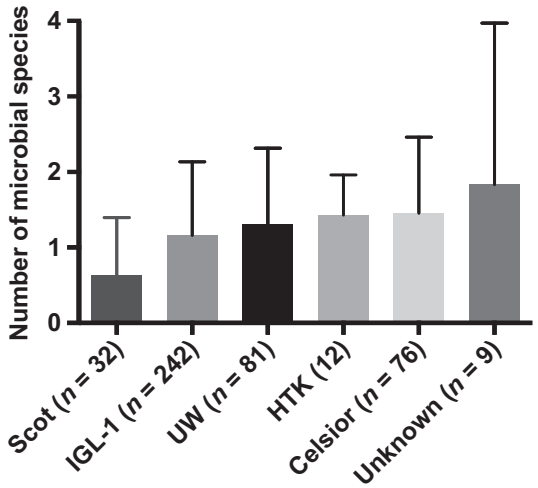

Figure 5 Number of microorganism(s) found in the pancreas preservation medium classified in function of the type of preservation solution used.

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 *S. 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%).

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.

Variables	Presence of microbial contamination in the preservation medium (n = 291)	Absence of microbial contamination in the preservation medium (n = 86)	P-value*
Pancreas weight, g	101.0 ± 24.7	88.3 ± 28.0	<0.001
Undigested tissue weight, g	16.4 ± 12.5	15.1 ± 11.3	0.365
Digested tissue weight, g	84.4 ± 23.4	73.2 ± 25.6	<0.001
Digestion rate, %	83.8 ± 11.7	83.1 ± 11.4	0.625
Digestion time, min	18.3 ± 4.1	18.8 ± 4.3	0.329
Tissue volume, ml	44.5 ± 16.4	37.1 ± 15.7	<0.001
Total number of islets, prepurification	374 391 ± 174 666	457 989 ± 819 814	0.107
IEQ prepurification	342 404 ± 185 906	345 774 ± 191 021	0.883
IEQ/per g pancreas (prepurification)	3529 ± 2018	4059 ± 2175	0.036
Mean prepurification islet size, µm	147.2 ± 69.3	144.2 ± 66.5	0.717
Embedded islets, %, prepurification	23.5 ± 20.4	23.8 ± 19.3	0.902
Fragmented islets, %, prepurification	12.8 ± 8.4	10.4 ± 5.2	0.021
Total number of islets postpurification	257 984 ± 158 424	289 808 ± 207 432	0.149
IEQ postpurification	245 597 ± 140 619	287 495 ± 168 973	0.028
IEQ/per g pancreas (postpurification)	2560 ± 1471	3139 ± 1851	0.004
Mean postpurification islet size, µm	155.6 ± 61.5	178.4 ± 98.9	0.014
Recovery rate, %	79.1 ± 42.7	86.0 ± 50.4	0.234
Isolation success (i.e., final yield ≥250 000 IEQ) (%)	133 (45.7)	44 (51.2)	0.069
Outcome			
Allotransplantation	131 (45.0)	45 (52.3)	<0.001
Autotransplantation	2 (0.7)	11 (12.8)	
Not transplanted	158 (54.3)	30 (34.9)	

IEQ, islet equivalent.

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

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

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

*Student t-test for continuous variables.

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.

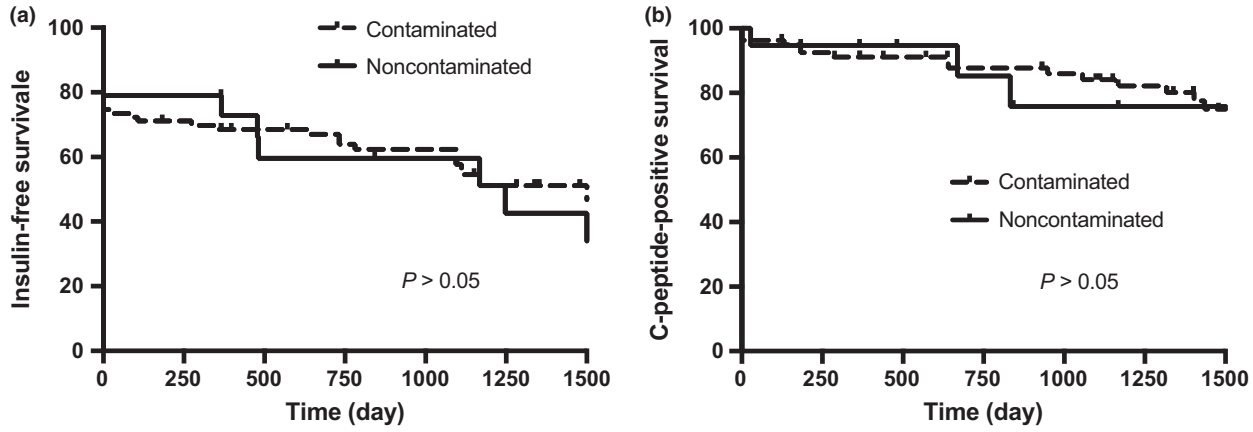

Figure 6 Survival curves for (a) insulin independence and (b) C-peptide positivity in preparation with and without preservation medium contamination (both *P* values >0.05). Gehan–Breslow–Wilcoxon test was used for survival curves comparison.

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 nonmicrobiological 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 4-year insulin independence rate compared to the other transplanted patients ($P = 0.853$).

In 2012, we implemented an additional microbiological sampling after 24 h of culture (sample B1). Over the period 2012–2016, 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 afterward. During the period 2006–2011, four patients were transplanted with culture-positive samples C preparations versus two during the period 2012–2016 (Fig. 7).

Table 5. Analysis of bacteriological samples collected from islet preparations accepted for further culture and transplantation throughout the processes of purification and culture.

	Islet preparations accepted for further culture and transplantation (n = 200)
Sample A (preservation medium)	
Positive (%)	140 (70.0)
Negative (%)	57 (28.5)
Not done (%)*	3 (1.5)
Sample B	
Positive (%)	1 (0.5)
Negative (%)	192 (96.0)
Not done (%)	7 (3.5)
Sample C (transplantation media)	
Positive (persistence) (%)	7 (3.5)
Positive (de novo) (%)	5 (2.5)
Negative (%)	186 (93.0)
Not done (%)	2 (1.0)
Gram staining (concomitant with sample C)	
Positive (%)	7 (3.5)
Negative (%)	190 (95.0)
Not done (%)	3 (1.5)
Transplanted preparations (%)†	192 (96.0)

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.

*Three preparations were transplanted without sample A being performed and were thus not counted in Tables 2–4.

†Six preparations were discarded due to positive Gram staining and two preparations were discarded for nonmicrobiological reasons.

Table 6. Islet preparations not transplanted due to positive Gram staining.

Preparation	Year	IEQ	Microbial contamination (Sample A, preservation medium)	Microbial contamination (Sample B)	Microbial contamination (Sample C, transplantation media)	Gram staining
A	2007	208 542	*Enterococcus* sp. *Staphylococcus aureus*	Negative	*Candida albicans*	Positive
B	2011	449 708	*Coagulase-negative staphylococci* *Candida albicans*	Negative	*Candida albicans*	Positive
C	2012	412 500	*Escherichia coli* *Enterococcus faecalis* *Proteus mirabilis* *Candida lusitaniae* *Candida robusta*	Negative	*Candida robusta* *Candida norvegensis*	Positive
D	2013	238 500	*Peptostreptococcus magnus*	Negative	*Candida robusta*	Positive
E	2013	266 444	*Citrobacter freundii* *Enterococcus faecalis* *Enterococcus faecium* *Klebsiella pneumoniae* *Staphylococcus aureus* *Candida robusta*	Negative	*Candida robusta*	Positive
F	2015	259 972	*Staphylococcus epidermidis*	Negative	*Candida albicans*	Positive

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 previously published rates varying between 19% and 68% [12–15,31]. 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 rates of microbial contamination of the preservation medium for other organs are as follow: 9–57% for kidneys [6,8], in 27% to 62% for livers [7,8], in 45–64% for heart valves [10], in 29% for lungs [9], and in 14–29% for cornea [11,32]. 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 exposer 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

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

Patient	Year	Age	Sex	IEQ	Type of graft	Microbial contamination (Sample A, preservation medium)	Microbial contamination (Sample B)	Microbial contamination (Sample C culture)	Gram staining (Sample C)	Therapy	Insulin independence (year)
A	2007	35.0	F	55 667	Autotransplantation	*Staphylococcus* sp. *Acinetobacter baumannii*	Negative	*Acinetobacter baumannii*	Negative	Standard antibiotic prophylaxis	9.2
B	2008	49.9	M	279 389	Allotransplantation	*Neisseria* sp. *Alpha-hemolytic Streptococcus* *Staphylococcus capitis*	Negative	*Staphylococcus capitis*	Negative	Standard antibiotic prophylaxis	0.0
C	2010	44.8	M	289 083	Allotransplantation	ND	Negative	*Candida kefyr*	Positive*	Fluconazol (15 days)	4.7
D	2011	56.7	F	262 542	Allotransplantation	*Candida albicans* *Streptococcus parasanguinis*	Negative	*Candida albicans*	Negative	Caspofungin and fluconazol (16 days)	3.7
E	2012	51.0	F	491 167	Allotransplantation	*Staphylococcus aureus* *Alpha-hemolytic Streptococcus* *Candida albicans*	Negative	*Candida robusta* *Candida norvegensis*	Negative	Fluconazol (10 days)	4.0
F	2013	63.1	F	320 667	Allotransplantation	Negative	Negative	*Pseudomonas stutzeri* *Paenibacillus* sp.	Negative	Standard Fluconazol (10 days)	1.3

ND, not done.

*Transplanted preparation without the information on Gram staining.

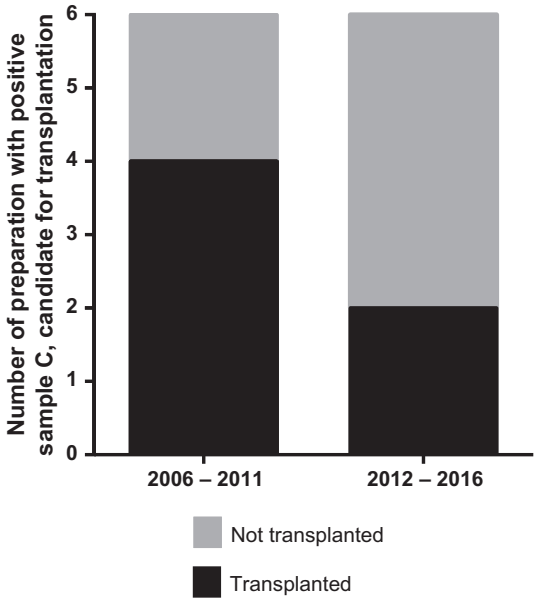

Figure 7 Number of preparations with culture-positive sample C that were processed in the view of transplantation during period 2006–2011 (no bacteriological sampling after 24 h of islet culture) and period 2012–2016 (bacteriological sampling after 24 h of islet culture: sample B1). Light gray: not transplanted;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

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 [13,31], or false negative culture of preservation medium.

The sterile technique during islet purification and culture continues to be of utmost importance. In a 10-year period, there were 6 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 24 h 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 [33]. Also, this may be of growing interest because of the current ongoing shift from brain-dead donors toward 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 autotransplantation setting, Jolissaint *et al.* [34] also found that bacterial contaminants in the final islet preparation were associated with lower islet yield and lower C-peptide/insulin independence rates in six patients. 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 strengthens 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 24 h should be performed as it further reduces the risk of contamination of the transplant product.

Authorship

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.

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Conflict of interest

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Complete list of all microorganisms found in the pancreas preservation media.

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