

Use of the BacT/Alert System for Rapid Detection of Microbial Contamination in a Pilot Study Using Pancreatic Islet Cell Products

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At the Islet Isolation Laboratory of the Scottish National Blood Transfusion Service, manual sterility testing data show that contamination rates are 57.7% for pancreas transport fluid, 4.3% for postpurification islet samples, and 0% for pretransplant islet samples. This pilot study presents the BacT/Alert System as an alternative to manual testing to provide more rapid and sensitive sterility results for islet cell products.

Islet transplantation is a minimally invasive procedure for the treatment of type 1 diabetes, which is characterized by recurrent severe hypoglycemia and glycemic unawareness. In this treatment, islets of Langerhans are isolated from donor pancreata and infused into the portal vein of a diabetic recipient. The Edmonton protocol for islet isolation and transplantation, published in 2000, demonstrated excellent patient outcomes following islet transplantation (1, 2). As a result, islet transplantation is now used worldwide as an alternative to whole-pancreas transplant for the treatment of severe, uncontrolled type 1 diabetes. Despite the widespread use of this treatment, little has been published on the microbial contamination rates of islet cell products and suitable methods for microbial detection prior to transplantation. Here we present microorganism contamination rates from the Scottish National Blood Transfusion Service (SNBTS) Islet Isolation Laboratory and compare standard manual sterility testing methods with results from the BacT/Alert rapid detection system. The rapid detection of microbial contamination is of benefit for islet transplantation because products are generally transplanted 24 to 48 h after isolation, before microbes can be detected by standard European Pharmacopoeia (EP) inoculation methods.

A total of 62 donor pancreata were processed at the SNBTS Islet Cell Laboratory between December 2009 and September 2012. The donor organs were used for isolation of islets using the Edmonton protocol (1). The following sample types were taken for sterility testing at various points during the procedure: pancreas transport fluid filtrate, pancreas transport fluid supernatant, postpurification islet supernatant, and pretransplant islet supernatant. A total of 52, 52, 46, and 21 samples were screened at each respective time point. Samples were inoculated into tryptic soy broth (TSB) and thioglycolate (Thio) medium (Cherwell Laboratories Ltd.), incubated for 14 days at 20 to 25°C and 30 to 35°C, respectively, and checked for signs of growth at 14 days. All broths were subcultured in a blind manner onto blood agar and Sabouraud-dextrose plates (Oxoid) and assessed for growth. Plates exhibiting growth were sent to the local clinical bacteriology laboratory for microbiological identification and antibiotic sensitivity testing.

The overall contamination rate for all samples was 40.4%. A total of 71.2% of pancreas transport fluid filtrate samples tested positive for contamination by microbes, compared to 57.7% of pancreas transport fluid supernatant samples (Table 1). Before islet isolation, the donor tissue is submerged briefly in povidone-

iodine antiseptic solution. This is effective at reducing microbial contamination, because only 4.3% of postpurification islet supernatant samples tested positive for microbial contamination (Table 1). Following purification, washing steps further reduce the microbial load, leading to a pretransplant contamination rate of 0%. These contamination rates are comparable to those reported in other studies (3, 4, 5, 6). Despite the low contamination rate of pretransplant samples in our study and others, there have been instances at other centers where contaminated islets have been transplanted into patients. This highlights the need for a rapid microbial detection method for testing of islet products (3).

In order to test whether the bioMérieux BacT/Alert 3D microbial detection system could be challenged to detect a low-level inoculum of microorganisms, pancreas transport fluid, postpurification supernatant, and pretransplant supernatant samples were spiked with microorganisms frequently found to contaminate islet products: *Staphylococcus epidermidis* (ATCC 12226; diluted 10^{-6}), *Candida albicans* (ATCC 10231; diluted 10^{-4}), and *Escherichia coli* (ATCC 8739; diluted 10^{-7}). The strains were obtained using Culti-Loops (Remel, Lenexa, KS). A single dilution of low-level inoculum (0.1 ml of 5 to 10 CFU/0.1 ml) was used to spike 0.5 ml of each sample. The spiked samples were then inoculated in BacT/Alert PF (aerobic) and BPN (anaerobic) culture bottles and loaded onto the BacT/Alert analyzer for 7 days or until detected as positive. PF and BPN culture bottles were chosen for this study because they have been previously validated by our organization as being effective at detecting low-level microbial growth in blood-related products and novel cell therapy products. Positive controls were prepared by inoculating 0.5 ml of 0.9% saline with 0.1 ml of the microbial dilutions for each islet sample type. The BacT/Alert PF culture bottles supported microbial growth in all islet samples (Table 2). There were no significant differences in

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TABLE 1 Microbes isolated from islet cell samples processed between December 2009 and September 2012 by the SNBTS Islet Isolation Laboratory

Parameter or microbe isolated	No. (%) of samples of the indicated type			
	Transport fluid filtrate	Transport fluid supernatant	Postpurification supernatant	Pretransplant supernatant
Total no. negative	15 (28.8)	22 (42.3)	44 (95.7)	21 (100)
Total no. positive	37 (71.2)	30 (57.7)	2 (4.3)	
Coagulase-negative <i>Staphylococcus</i> sp.	7 (13.5)	7 (13.5)	2 (4.3)	
Yeasts	7 (13.5)	3 (5.8)		
<i>Escherichia coli</i>	4 (7.7)	5 (9.6)		
<i>Candida albicans</i>	1 (1.9)	1 (1.9)		
<i>Pseudomonas fluorescens</i>	5 (9.6)	1 (1.9)		
<i>Staphylococcus epidermidis</i>	3 (5.8)	2 (3.8)		
<i>Stenotrophomonas maltophilia</i>	5 (9.6)			
<i>Lactobacillus</i> sp.	2 (3.8)	2 (3.8)		
<i>Pseudomonas</i> sp.	1 (1.9)	3 (5.8)		
<i>Staphylococcus aureus</i>	3 (5.8)	1 (1.9)		

detection time between spiked islet samples and positive controls. Use of BPN bottles resulted in detection within the culture period (168 h) in all but two cases and a significant difference in detection time in one case (Table 2). These data show that the BacT/Alert system is capable of detecting low-level contaminants in multiple islet cell sample types.

Nonspiked islet samples were tested by the BacT/Alert system, and parallel sterility testing was carried out using EP methods as described above. Organisms were identified by subculture onto blood agar plates and identification by the local microbiology reference laboratory using the bioMérieux Vitek system. Table 3 shows a comparison of the organisms that were isolated by the manual testing method and the BacT/Alert method. While both methods detected contamination in transport fluid samples, the organisms identified differed between the two techniques, with

the BacT/Alert system detecting a larger number of microorganisms. In addition, only the BacT/Alert system detected contamination in postpurification samples. Neither method detected contamination in pretransplant samples, consistent with the low contamination rates observed in these products. These results demonstrate that the BacT/Alert system has a higher sensitivity than the manual method currently used by the SNBTS laboratory, a finding supported by data from Khuu et al. (7).

This study and others (3, 5, 6, 7, 8) have shown the occurrence of microbial contamination in islet cell products, including a high incidence of contamination in organ transport fluid. Pancreata are retrieved from deceased donors during multiorgan procurement and packaged for islet isolation in manners identical to those distributed for whole-organ transplant. Therefore, we estimate that over 50% of pancreata distributed for whole-organ transplant

TABLE 2 Microbial detection in spiked islet samples and corresponding saline controls by the BacT/Alert system using PF (aerobic) and BPN (anaerobic) bottles^a

Organism	Sample type	BacT/Alert PF culture bottle		BacT/Alert BPN culture bottle	
		Detection time (h) (mean ± SD)	<i>P</i> value	Detection time (h) (mean ± SD)	<i>P</i> value
<i>S. epidermidis</i>	Transport fluid	29.3 ± 7.4	0.3210	17.4 ± 2.5	0.2839
	Saline (transport fluid control)	34.2 ± 1.3		19.2 ± 0.6	
	Postpurification	32.6 ± 0.9	0.6218	19.9 ± 1.4	0.5956
	Saline (postpurification control)	34.3 ± 3.9		19.4 ± 0.7	
	Pretransplant	26.9 ± 0.4	0.0528	Negative	—
	Saline (pretransplant control)	34.7 ± 3.5		19.1 ± 1.0	
<i>C. albicans</i>	Transport fluid	21.0 ± 7.1	0.2584	16.1 ± 2.1	0.0023
	Saline (transport fluid control)	54.9 ± 43.9		26.3 ± 1.5	
	Postpurification	27.8 ± 0.5	0.3466	26.6 ± 2.7	0.8149
	Saline (postpurification control)	43.2 ± 24.7		26.2 ± 1.8	
	Pretransplant	29.1 ± 0.9	0.2421	25.3 ± 0.2	0.8249
	Saline (pretransplant control)	31.5 ± 2.9		25.5 ± 2.0	
<i>E. coli</i>	Transport fluid	12.9 ± 0.3	0.3635	11.7 ± 0.3	1.0000
	Saline (transport fluid control)	13.4 ± 0.7		11.7 ± 0.5	
	Postpurification	13.7 ± 0.7	0.4068	11.7 ± 0.5	0.5979
	Saline (postpurification control)	14.2 ± 0.5		11.9 ± 0.2	
	Pretransplant	15.3 ± 0.6	0.0761	Negative	—
	Saline (pretransplant control)	14.2 ± 0.4		12.1 ± 0.1	

^a *n* = 3 for all samples; *P* values are from an unpaired *t* test.

TABLE 3 Comparison of positive results obtained from a manual testing method and the BacT/Alert system

Sample type (total no. screened)	Organism detected	No. (%) of positive donor samples identified by:		
		Manual testing	BacT/Alert PF	BacT/Alert BPN
Transport fluid (3)	Coagulase-negative <i>Staphylococcus</i> sp. ^a	2 (66.7)		
	<i>Staphylococcus capitis</i>	2 (66.7)		
	<i>Staphylococcus warneri</i>	1 (33.3)	2 (66.7)	1 (33.3) ^b
	<i>Staphylococcus aureus</i>			1 (33.3)
	<i>Streptococcus mitis</i> / <i>Streptococcus oralis</i>			1 (33.3)
	<i>Candida albicans</i>			1 (33.3)
	<i>Pseudomonas fluorescens</i>			1 (33.3)
	<i>Bacillus</i> sp.			1 (33.3)
Postpurification (3)	<i>Staphylococcus capitis</i>			1 (33.3)
	<i>Micrococcus</i> sp.			1 (33.3) ^c
Pretransplant (4)	None			

^a Both donor samples from which coagulase-negative *Staphylococcus* species were manually isolated were also positive by the BacT/Alert system, but the organism was identified fully as *Staphylococcus warneri*.

^b The donor sample from which *Staphylococcus warneri* was isolated in a BacT/Alert BPN bottle was also detected in a BacT/Alert PF bottle.

^c *Micrococcus* sp. was isolated from a subculture of a BacT/Alert bottle that had been classified as negative.

are likely to be transported in microbially contaminated transport fluid. This conclusion is supported by internal data and data from other centers, which show that contaminants are commonly found in transport fluid following heart, kidney, or liver procurement (9, 10, 11). Because islet cells are normally transplanted 24 to 48 h after isolation, rapid methods of microbial detection would benefit islet transplant programs by identifying potentially hazardous contaminants in transport fluid and postpurification samples that would preclude transplantation of the product or by allowing earlier intervention in the event that a contaminated product was transplanted. This pilot study shows that the BacT/Alert 3D microbial detection system is capable of rapidly detecting low-level contamination in islet cell products and detects a larger range of contaminants than that detected by manual methods of sterility testing. Therefore, islet isolation laboratories should consider validation of this automated method for sterility testing of islet cell products. Although the procedures for islet isolation are largely similar across centers internationally, currently there are no standardized release criteria for islet cell products. We suggest that sterility testing using the BacT/Alert system could be used, together with assays of islet quality, to ensure that islet products are released for transplantation in a consistent and safe manner.

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