QR-QA-013-01: IIDP Pancreas Decontamination Protocol for Islet Isolation Centers

1) **Objective**

To provide guidance to IIDP Islet Isolation Centers' (IICs) staff on the main steps that are required by the IIDP to ensure that proper procedures are taken to decontaminate the pancreas prior to beginning an IIDP islet isolation.

a) This protocol is a summary of best practices that have been published by experienced islet isolation centers or have been relayed to the IIDP from current IICs.

2) **Important Points to Consider**

a) Use of a surface disinfectant is an important first step in reducing contamination of the pancreas.

b) Use of broad-spectrum antibiotics and antimycotics/antifungals in the rinse solutions can help to reduce a possible bioburden from the organ procurement process and therefore reduce the chance of contamination of the final islet preparation.
   i) Antibiotics chosen must be effective against both gram positive and gram-negative bacteria such as ciprofloxacin (10mg/mL) or a combination of cefazolin (1mg/mL) and gentamycin (0.3mg/mL).
   ii) Antimycotics/antifungals must be effective for mold, fungal, and yeast contaminants such as Amphotericin B (250ug/mL) or Nystatin (30 units/mL).

c) Multiple rinses will physically reduce the chance of contaminated entities passing from the pancreas to the islet isolation process.

d) Once the pancreas has been decontaminated, be sure to use new, sterile instruments, containers, and gloves to reduce re-contamination in the procedure.

e) Note that there is a high probability that if fungal or yeast contamination is introduced during the procurement process, it will not be rinsed away during the isolation process like most bacterial contamination, nor removed during the purification, therefore the decontamination process and the addition of antifungal/antimycotic solutions are very important to the procedure.

3) **Procedures**

a) Prepare solutions needed for decontamination each in 500 mL Nalgene jars or beakers:
   i) Decontamination Solution #1 – 150 mL of Providone-iodine and 150 mL of HBSS
      (1) 1% chlorhexidine digluconate or a similar surface antiseptic can be substituted.
   ii) Decontamination #1 Rinse – 200 mL of HBSS
   iii) Decontamination Solution #2 – 250 mL of HBSS with 0.25mL Ciprofloxacin (10mg/mL) for both gram-positive and gram-negative bacteria, 2.5 mL of Amphotericin B (250ug/mL) for fungal, mold, and yeast contamination
      (1) Other broad-spectrum antibiotics may be substituted for Ciprofloxacin: (1 mg/mL Cefazolin for gram positive bacteria plus 0.3mg/mL gentamicin for gram negative bacteria).
      (2) Other antimycotic/antifungal solutions may be substituted for Amphotericin B: (30 units/mL Nystatin).
   iv) Decontamination Solution #3 – 250 mL of HBSS (in 500 mL Nalgene with lid for transfer to a new hood)

b) Open shipping box from OPO and remove pancreas container and place under original trimming hood.
   i) Open shipping container and transfer the pancreas to the pancreas trimming pan.
   ii) Pour the procurement solution into the trimming pan to just cover the pancreas in trimming hood. Verify that the gland is acceptable for islet isolation. Keep pan cold by placing on a larger pan with sterile ice.

c) Begin the gross trimming process. Remove the duodenum, spleen and gross extraneous tissue.
   i) Replace duodenum in the original shipping container until determined if it is needed for non-islet biospecimens (NIB) shipment.
   ii) Place spleen in a 50 mL conical and refrigerate until determined if needed for NIB shipment.
   iii) Remove remaining trimmed tissue from the hood and dispose.
   iv) Pass under the hood the decontamination solutions and containers.

d) Using the trimming forceps, dip grossly trimmed pancreas into #1 Decontamination Nalgene jar containing a cold 50% Betadine (Providone-iodine) 150 mL/HBSS 150 mL solution and agitate in solution for 30-60 seconds.
   i) This decontamination solution can be replaced using a qualified substitute as listed above.

e) Carefully lift pancreas using decontaminated forceps above the first jar and allow betadine solution to drip as an additional 200 mL of cold HBSS is poured over the pancreas to give a first rinse.
   i) Rinses are important to help remove any contamination and remove excess antiseptic.

f) Transfer pancreas to #2 Decontamination Nalgene jar containing cold 0.25 mL Cipro, 2.5 mL Amphotericin, and 250 mL of HBSS. Soak for up to 3 minutes.
   i) Antibiotics and antimycotics can be substituted with other appropriate solutions as listed above.

g) Transfer pancreas to #3 Decontamination Nalgene jar with lid, containing 250 mL of cold HBSS for third rinse.
   i) Agitate in rinse solution for 30 seconds.

h) Disinfect the outside of #3 Decontamination Nalgene jar and move to a new hood for final trimming and canulation with new gloves, instruments, trimming pan, and solution.
   i) If a new hood is not available, set #3 jar with pancreas in sterile ice outside of the hood, thoroughly clean the trimming hood, and stock with new sterile instruments, trimming pan and trimming solution. Disinfect #3 jar and return to cleaned hood and continue with final trimming and canulation.

4) **References**

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b) David W. Scharp [1, 2, *], Jayagowri Arulmoli [1], Kelly Morgan [3], Hannah Sunshine [4], Ergeng Hao [1]. Advances in Human Islet Processing: Manufacturing Steps to Achieve Predictable Islet Outcomes from Research Pancreases. *OMB Transplantation,* 2019; 3,1; DOI:10.21926/obm.transplant.1901052

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d) Camillo Ricordi,[1] Julia S. Goldstein,[2] A.N. Balamurugan,[3]*et al.* National Institutes of Health–Sponsored Clinical Islet Transplantation Consortium Phase 3 Trial: Manufacture of a Complex Cellular Product at Eight Processing Facilities. *Diabetes,* 2016;65:3418-3428. DOI: 10.2337/db16-0234