ASSESSMENT OF HUMAN ISLET COMPOSITION AND PURITY BY IMMUNOFLUORESCENCE STAINING

I. Definitions

1. **Cryosections:** Sections of a tissue/cells embedded in optimal cutting temperature (OCT) compound and frozen -80ºC.

2. **Indirect Immunofluorescence Staining:** Immunohistochemical procedure based on antigen detection by fluorescence in histological sections using a combination of primary and secondary antibodies where the primary antibody is directed to the antigen of interest and the fluorescently conjugated secondary recognizes species where the primary antibody was raised. Histological sections are viewed using a microscope system equipped with an appropriate light source and filter set to allow for visualization of fluorescence tissue staining.

II. Equipment and Materials

1. Equipment
    - 4°C Refrigerator (ArcticTemp)

2. Supplies and Materials
    - PBS (phosphate buffered saline) with no Ca/Mg, 1X (Invitrogen 14190-144)
    - BSA (bovine serum albumin, Sigma A-6003)
    - SlowFade Gold (Molecular Probes S36938)
    - Triton-X-100 (BioRad 1610407)
    - Normal Donkey Serum (NDS, Jackson Immuno Research 017-000-121)
    - 4',6-diamidino-2-phenylindole (DAPI, ThermoFisher Scientific D1306)
    - Kartell Staining Chambers (VWR 25460-907)
    - PAP Marker (Research Products International 195506)

III. Preparation of Reagents for Immunofluorescence Staining of Islet Cryosections

1. **10% Triton-X-100 stock (30 mL)** – combine 3 mL Triton-X-100 and 27 mL 1X PBS. Mix on shaker for 30 min or until Triton-X-100 is completely dissolved and store at 4ºC for up to 1 month.

2. **Permeabilization Solution (0.2% Triton, 50mL)** – combine 1 mL of 10% Triton stock and 49 mL 1X PBS.

3. **Blocking Buffer (5% NDS, 4 mL)** – combine 0.2 mL NDS and 3.8 mL 1X PBS.

4. **Antibody Buffer (10mL)** – combine 0.1 g BSA, 0.1 mL 10% Triton Stock, and 9.8 mL 1X PBS.

5. **DAPI staining solution (1:25,000, 50 mL)** – combine 2 µL DAPI stock (5mg/mL) and 50 mL 1X PBS.

IV. Immunofluorescence Staining Procedure on Islet Cryosections

1. Use freshly-made antibody incubation buffers, and wash buffers. Steps 3, 4, 8, 10, 11 can be done in Kartell Staining Chambers.

2. Let the frozen sections thaw at room temperature and air-dry for about 30 minutes.

3. Wash the sections with 50 mL 1X PBS 3 times for 5 min to remove the OCT.

4. Permeablize the tissue section with 0.2 % TritonX -100 for 15 minutes at room temperature. Wash the tissue in 50 mL 1X PBS 3 times for 3-5 min.

5. Draw circles or rectangles around the sections with PEP marker and let them dry for about 5 minutes.

6. Block the sections with 5% normal donkey serum (made from 100% stock)/1X PBS at room temperature for 90 min in a humidified chamber.

7. Aspirate the blocking solution, add primary antibodies (**Table 1**) diluted in 0.1% Triton-X-100 (made from 10% Triton-X-100 stock)/1% BSA/1X PBS and incubate in a humidified chamber overnight at 4ºC.

8. Aspirate the primary antibodies and wash the sections with 1X PBS three times for 15 min/each.

9. Add secondary antibodies (**Table 1**) diluted in 0.1% Triton-X-100/1% BSA/1X PBS and incubate for 1.5 hours at room temperature in a humidified chamber.

10. Aspirate the secondary antibody and counterstain slides with 1:25,000 DAPI/PBS for 10min at room temperature.

11. Remove from DAPI and wash the sections with 1X PBS three times for 5 min. each.

12. Mount the sections with SlowFade Gold mounting medium.

V. Imaging and Analysis of Fluorescently Labeled Islet Cryosections

1. Capture images of islet sections using a high-resolution whole slide scanning system (ScanScope FL, Aperio/Leica) connected to a web-based digital slide repository powered by eSlide Manager and housed in the Vanderbilt University Medical Center data center (*examples of islet images are shown in **Figures 1** and **2***).

2. Using a tissue classifier algorithm (Halo™, Indica Labs) analyze islet images (50 -100 islets/labeling experiment) to provide a quantitative assessment of the islet cell composition and purity for a given human islet preparation (*examples of quantitative islet assessment are shown in the legend of **Figures 1** and **2***).

VI. Data Storage and Reporting

1. Store the data in the appropriate server location(s).
2. Annotated images may be uploaded to the HPAP database
3. Document any deviations from this protocol that occurred.

Table 1. List of primary and secondary antibodies for -assessment of islet cell composition and islet purity

Primary Antibody	Vendor	Product number	Final Dilution	Secondary Antibody	Vendor	Product number	Final Dilution
C-peptide (rat)	Developmental Studies Hybridoma Bank	GN-ID4 RRID:AB_2631151	1:100	Rat IgG-Cy2 (donkey)	Jackson ImmunoResearch	712-225-150 RRID:AB_2340673	1:500
				Rat IgG-Cy5 (donkey)	Jackson ImmunoResearch	712-175-150 RRID:AB_2340671	1:200
Glucagon (mouse)	Abcam	ab10988 RRID:AB_297642	1:250	Mouse IgG-Cy3 (donkey)	Jackson ImmunoResearch	715-165-150 RRID:AB_2340813	1:500
Glucagon (rabbit)	Cell Signaling Technology	2760S RRID:AB_10698611	1:100	Rabbit IgG-Cy5 (donkey)	Jackson ImmunoResearch	711-175-152 RRID:AB_2340607	1:200
Somatostatin (goat)	Santa Cruz Biotechnology	sc-7819 RRID: AB_2302603	1:500	Goat IgG-Cy5 (donkey)	Jackson ImmunoResearch	705-175-147 RRID:AB_2340415	1:200
HPX1 (mouse)	Gift from P. Streeter, Oregon Health and Science University	not applicable RRID:AB_2631151	1:100	Mouse IgG-Cy3 (donkey)	Jackson ImmunoResearch	715-165-150 RRID:AB_2340813	1:500

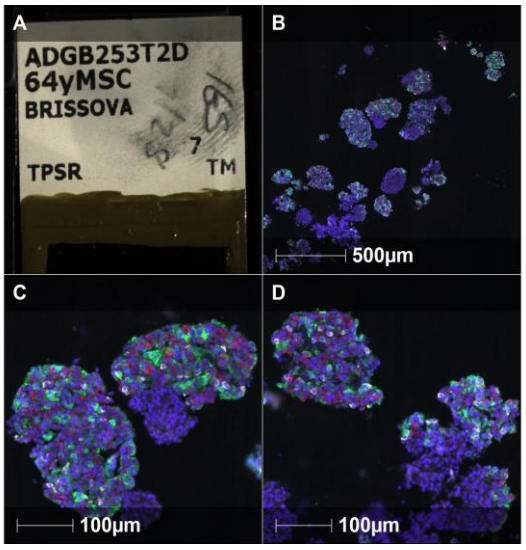

Figure 1. Histological assessment of human islet composition. Human islet preparation was processed for cryosections. (**A**) Each specimen label is captured by slide scanner and contains information about UNOS ID, disease type, donor age, gender, and islet isolation center. (**B - D**). Islet composition was determined by immunofluorescence for islet markers (β cells – C-peptide, green; α cells – glucagon, red; δ cells – somatostatin; white, DAPI – blue) using a tissue classifier algorithm (Halo™). **This islet preparation contained 35% α cells, 50% β cells, and 15% δ cells**.

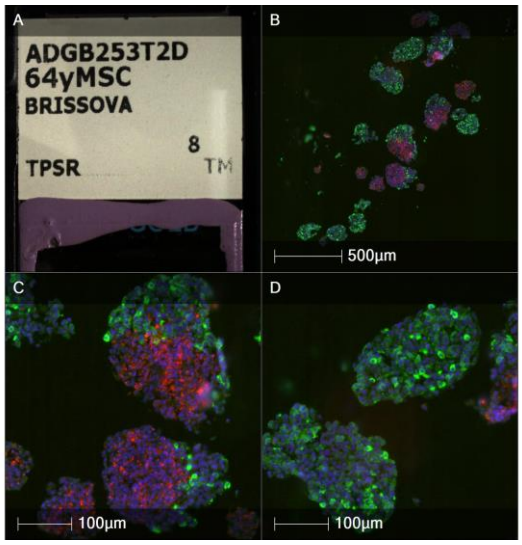

Figure 2. Histological assessment of human islet purity. Human islet preparation was processed for cryosections. (**A**) Each specimen label is captured by slide scanner and contains information about UNOS ID, disease type, donor age, gender, and islet isolation center. (**B - D**) Sections were labeled for islet markers (C-peptide, glucagon, and somatostatin – green), acinar cell marker Hpx1 (red), counterstained with DAPI (blue) and then analyzed with algorithm (Halo™) to measure islet purity. **This islet preparation had 67% purity.**

VII. References

1. Dai C, Brissova M, Hang Y, Thompson C, Poffenberger G, Shostak A, et al. Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. Diabetologia. 2012 Mar;55(3):707–18. PMCID: PMC3268985
2. Guo S, Dai C, Guo M, Taylor B, Harmon JS, Sander M, et al. Inactivation of specific β cell transcription factors in type 2 diabetes. J Clin Invest. 2013 Aug;123(8):3305–16. PMCID: PMC3726150