Qualitative Assessment of Islet Viability by Staining with Fluorescein Diacetate (FDA) and Propidium Iodide (PI) Dyes

I. Definitions

1. **Actual Islets (AI):** The actual number of islets counted.
2. **Islet Equivalent (IEQ):** An islet is quantified as 150 µm diameter by mathematically compensating for the volume of the islet.
3. **Fluorescein Diacetate/Propidium Iodide (FDA)/(PI) Viability Assay**: It is a rapid fluorometric method to test the integrity of the plasma membrane simultaneously using inclusion and exclusion dyes; the assay differentiates between viable and nonviable cells and is, consequently, used for determination of viability of islet preparations.
    - The inclusion dye is fluorescein diacetate (FDA) and the exclusion dye is propidium iodide (PI). The final concentrations are as follows:
        - FDA: 0.46 µM
        - PI: 14.34 µM
    - Fluorescein diacetate is a nonpolar ester, which passes through plasma membranes and is hydrolyzed by intracellular esterases to produce free fluorescein. The polar fluorescein is confined within cells with an intact plasma membrane and can be observed under appropriate excitation conditions. FDA functions as an inclusion dye, i.e., viable cells will appear bright green fluorescent using FDA.
    - Propidium iodide functions as an exclusion dye that cannot penetrate living cells but readily enters dead or dying cells. Once PI penetrates through the cell membrane, it binds to nucleic acids and causes them to fluoresce bright orange/red. PI absorbs green light and fluoresces orange/red.

II. Equipment and Materials

1. Equipment
    - Fluorescent Microscope
    - Calculator or computer software (e.g. Excel) with the mean and standard deviation functions
2. Supplies and Materials
    - Fluorescein diacetate, stock solution 24 µM (9.9 µg/mL in acetone), Sigma, Cat. #F-7378, or equivalent
    - Propidium iodide, stock solution 750 µM (0.5 mg/mL in DPBS, pH approximately 7.4), Sigma, Cat. #P-4170, or equivalent
    - Acetone, Sigma, Cat. #179124, or equivalent
    - Sterile 10 x 35 mm cell culture dishes, Nunc Cat. #174926, or equivalent

- DPBS (Dulbecco's Phosphate Buffered Saline) without calcium or magnesium, Mediatech, Part #21-031, or equivalent
- Pipets: 200 and 1000 µL with associated tips (P-200 ART and P-1250 ART)

III. Procedures

1. Limitations
    - Once the dye is added to the islets, the assessment must take place as quickly as possible. If there is a delay of more than 15 minutes, the accuracy of the assessment will be diminished as the islets lose their viability with time.
    - Both of the fluorescent dyes used in this assay are light sensitive and must be kept in the dark, covered with aluminum foil.
    - The fluorescent dyes are temperature sensitive and must be stored as follows:
        - FDA: ≤ -20°C
        - PI: 2-8°C
2. Assay Set Up
    - Assemble all items described in the "Supplies and Materials" section.
    - Prepare Fluorescent dyes: Fluorescein Diacetate (FDA) and Propidium Iodide (PI).
3. Preparation of Fluorescein Diacetate and Propidium Iodide

    A. Stock Fluorecein Diacetate (FDA) Solution 24 µM

    a. Calculation:

    - FDA FW = 416.4
    - Stock Concentration = 24 µM
    - Volume required = 2mL

FW x Concentration x Volume =
$(416.4) \times (24 \times 10^{-6}) \times (200 \times 10^{-3}) = 0.00199g$ FDA

Components	Quantity to be Added	Quantity added	Supplier & Lot Number	Expiration Date
FDA	0.00199 g	g		
Acetone	200 mL	mL		

- Store Stock FDA solution at ≤ -20°C for up to six months.
- Cover it with aluminum foil, as the dye is light sensitive.
- Label with assigned lot number, date of expiration and initials of preparer.

- Assigned Lot Number: _____
- Prepared by: _____ Date: _____
- Reviewed by: _____ Date: _____

B. Stock Propidium Iodide (PI) Solution 750 µM

a. Calculation:

- PI FW = 668.4
- Stock Concentration = 750 µM
- Volume Required = 25 mL

FW x Concentration x Volume =
(668.4) x (750 x 10^{-6}) x (200 x 10^{-3}) = 0.0125 g PI

Components	Quantity to be Added	Quantity added	Supplier & Lot Number	Expiration Date
PI	0.0125 g	g		
DPBS	25 mL	mL		

- Store Stock PI solution at 2 to 8°C for up to six months.
- Cover it with aluminum foil, as the dye is light sensitive.
- Label with assigned lot number, date of expiration and initials of preparer.
  - Assigned Lot Number: _____
  - Prepared by: _____ Date: _____

- Check the required amount of each dye necessary to make up indicated stock solutions (see above). Remove FDA from the freezer (≤ - 20°C) and PI from the refrigerator (2-8°C). Weigh the required amount of the reagent on an analytical balance.
- Dissolve 0.00199 g of FDA in 200 mL of acetone in a glass bottle and cover with aluminum foil. Store in 500 µL aliquots at -20°C.
- Dissolve 0.0125 g of PI in 25 mL of DPBS and cover with aluminum foil. Store in 100 µL aliquots at 2-8°C.
- Discard used stain.
- Record the expiration date of each solution. The expiration date, for both PI and FDA stains, is six months from the date of preparation.
- Record the lot numbers of all the reagents used in the preparation of dyes.

IV. Staining and Estimation of Viability

1. Add 400 µL islet suspension to a 48-well plate.
2. Quickly add first 8 µL of PI and then 8 µL of FDA to the islet suspension. Gently swirl to thoroughly mix.
3. Incubate in the dark for up to 15 min.
4. Assess the preparation immediately using the fluorescent microscope. Image islets using an Olympus SZX12 stereomicroscope system. Capture multiple fields ensuring that FDA/PI staining is visualized in 50 – 100 islets per preparation.

Note: FDA produces bright green fluorescence in viable cells, while PI produces red fluorescence in dead or dying cells.

V. Interpretation of Results

1. The FDA freely passes through the cell membrane of live cells. Viable cells appear bright fluorescent green when stained with FDA. In a live cell, FDA is hydrolyzed to the polar free fluorescein, and it is trapped within the intact membranes of the viable cells present in islets.
2. The PI stains the nuclei of dead/non-viable cells only. Dead cells appear bright fluorescent red/orange. PI does not cross the membrane of viable cells.
3. FDA/PI staining captures viable and dead cells in both islets and residual exocrine tissue. This staining provides a rapid qualitative viability assessment of human islet preparation, however the 3-D nature of islets and exocrine tissue remnants precludes accurate cell counting. The Vanderbilt HIPP will supplement viability assessment with counts of live and dead cells in a single cell suspension using Countess II technology (Life Technologies AMAX1000) (see HIPP-04-v01 Quantitative Assessment of Islet Viability by Trypan Blue).

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.

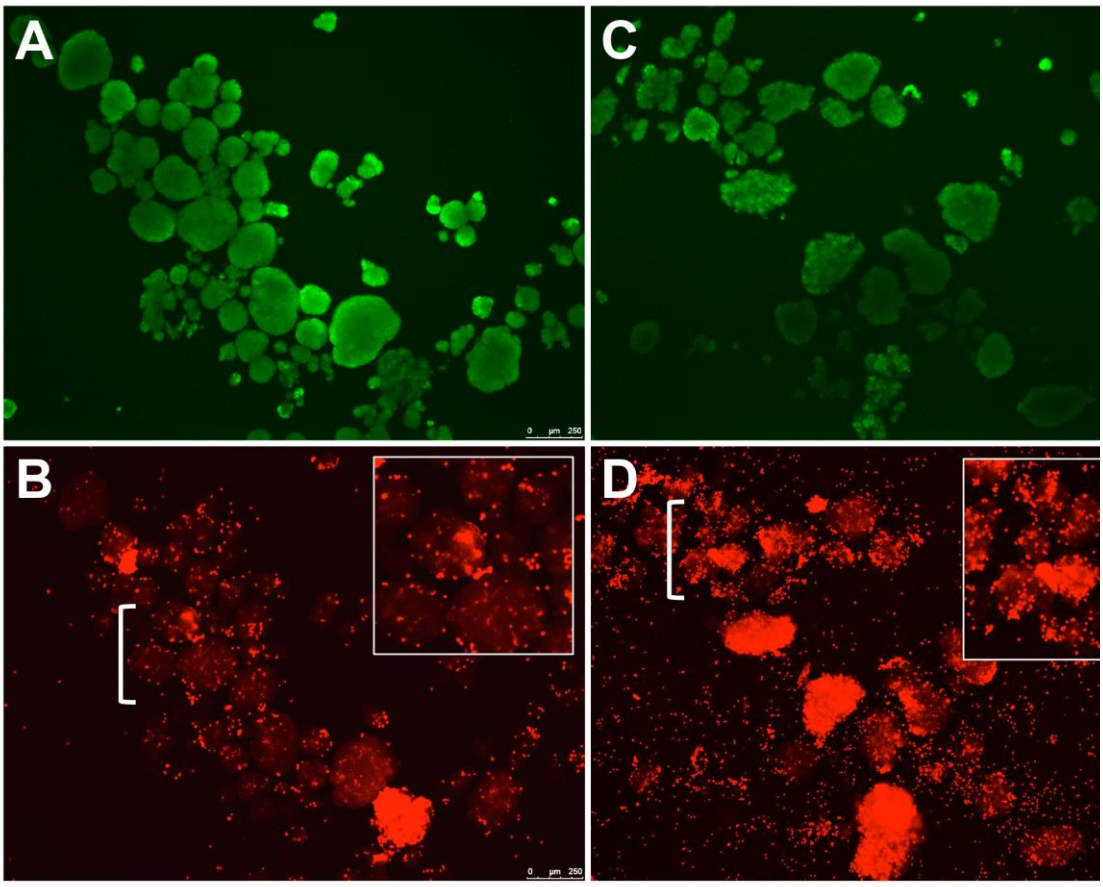

Figure 1. Qualitative viability assessment of human islet preparations by FDA/PI staining. Two human islet preparations; preparation #1 (**A**, **B**) and preparation #2 (**C**, **D**) were stained side-by-side with FDA and PI dyes. FDA staining (green) appeared

References

1. Bank, HL (1987). Assessment of Islet Cell Viability Using Fluorescent Dyes. Diabetologia, 30:812-816. Bank, HL (1988).

2. Rapid Assessment of Islet Viability with Acridine Orange and Propidium Iodide. Invitro Cellular & Developmental Biology, 24:4, pp. 266-273.

3. Ricordi, C. Pancreatic Islet Cell Transplantation. Austin: R.G. Landes Company, 1992:137-138.