Quantitative Assessment of Islet Viability by Staining with Trypan Blue Dye

I. Definitions

1. Islet Equivalent (IEQ): An islet with a diameter of 150 μm determined mathematically by compensating for islet shape.
2. Trypan Blue stain: A vital stain used to selectively color dead tissues or cells blue. Trypan Blue is a diazo dye. In a viable cell Trypan Blue is excluded, however, it traverses the membrane in a dead cell (similar to propidium iodide). Hence, dead cells have a distinctive blue color under a microscope.

II. Equipment and Materials

1. Equipment
   - Centrifuge (Eppendorf 5702R)
   - Countess II (Life Technologies AMAX1000)
2. Supplies and Materials
   - 1X Phosphate buffered saline without Ca/Mg (PBS, Invitrogen 14190-144)
   - 0.5 M Ethylenediaminetetraacetic acid, pH8.0 (EDTA, Cellgro 46-034-Cl)
   - Accutase (Innovative CellT AT-104)
   - CMRL1066 (Cellgro 15-110-CV)
   - L- Glutamine (Invitrogen 25030-081)
   - Penicillin/Streptomycin (Invitrogen 15140-122)
   - Fetal Bovine Serum (FBS Millipore TMS-013-B)
   - Trypan Blue (Invitrogen T10282)
   - Chamber slide (Invitrogen C10283)
   - 1. 5ml Eppendorf tubes (Denville C-2171)

III. Procedures

1. Dispersion of islets into single cell suspension
   - Prepare 2 aliquots of approximately 150 IEQs of handpicked islets in 1.5 mL tubes to determine viability of islets. Note: This is important especially for islet preparations with lower purity where cell death in the contaminating exocrine compartment may occur at higher rate compared to islets and thus could lead to underestimating islet viability.
   - Wash islets with 2mM EDTA made in 1X PBS and centrifuge them at 1000 rpm for 1 minute at 4°C. Repeat two times.
   - After last wash, resuspend the pellet in 500 μL of Accutase.
   - Triturate islets for 10 minutes at 37°C using a multichannel pipet with 200 μL tips. Note: As islets are getting dispersed, the sample should become slightly opaque.
   - Quench the reaction with 500 μL of CMRL-1066 media containing 10% FBS, 2 mM L-glutamine, 1% penicillin/streptomycin.

- Rinse the cells with 1 ml of CMRL-1066 medium, centrifuge at 1800 rpm for 3 minutes at 4°C. Carefully remove the media without disturbing the pellet at the bottom of the tube. Repeat one more time.
- Resuspend the cell pellet in 100 μL of CMRL-1066 medium by gently flicking the tube and keep the sample on ice.

2. Cell Counting

- From each islet sample, transfer two 15 μL aliquots of the cell suspension into 1.5 ml tubes. Mix well before pipetting the sample.
- Prepare the cell counting chamber slide for sample loading (slide has two chambers and looks similar to the hemocytometer but without grids).
- Gently mix the first aliquot of the islet cell suspension with 15 μL of Trypan Blue dye and then load 10 μL of the mixture into the chamber slide.
- Wait approximately 20 seconds for the cells to spread out on a slide, insert the slide into the Countess II cell counter and acquire cell counts following instrument guidelines. The output of cell and viability counts for a cell suspension of purified islets is illustrated in **Figure 1**.
- Repeat cell counts for the second aliquot of the same islet sample using the second slide chamber.
- Repeat the highlighted steps (bullets 2 – 5) for the remaining sample.
- Note: Cells need to be imaged within 15 minutes of Trypan Blue addition due to the dye cytotoxicity.
- Report average values of viable cells for duplicates of purified islets based on two cell counts per each replicate.

IV. Data Storage and Reporting

1. Store 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.

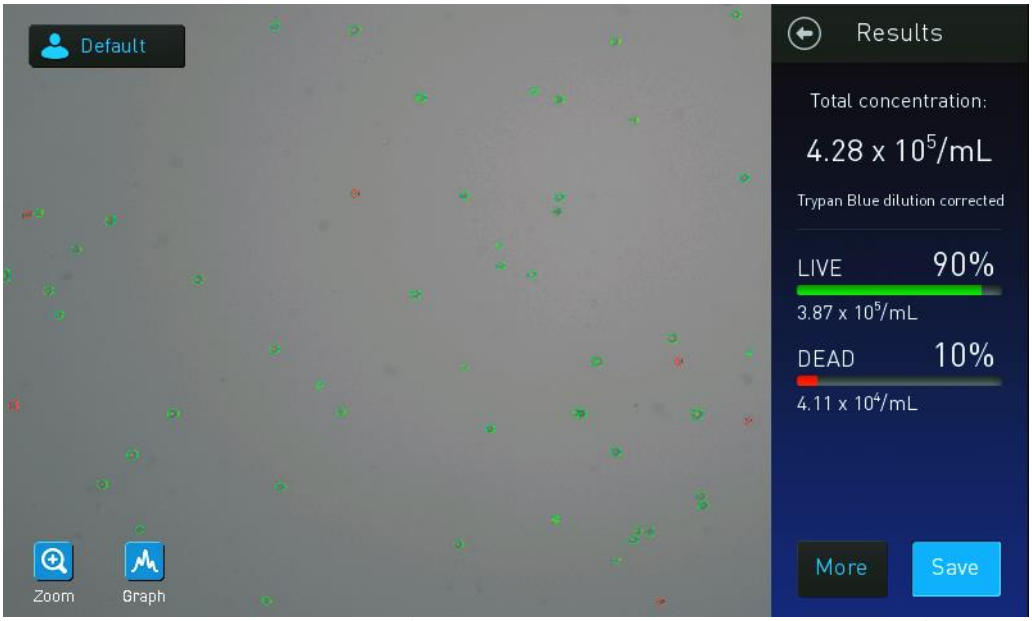

Figure 1. Quantitative viability assessment of human islet preparations by Trypan Blue staining. Purified human islets were dispersed with Accutase. Cell suspension was labeled with Trypan Blue and viability measured using Countess II automated cell counter. The output image of the cell counter on the left shows viable cells in *green* and dead cells in *red*. The total cell concentration and viability are reported in the panel on the right-hand side.

V. References

1. Louis KS, Siegel AC. Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods. Mammalian Cell Viability …. Totowa, NJ: Humana Press; 2011. p. 7–12.

2. Bansal H, Yihua Q, Iyer SP, Ganapathy S, Proia DA, Proia D, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. Leukemia. 2014 May;28(5):1171–4. PMCID: PMC4369791