Successful Clinical Islet Isolation and Purification using a Bovine Product-free GMP Collagenase and Neutral Protease

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Bovine Product-Free Serva NB1 Collagenase and Roche Liberase have a similar HPLC Profile

University of California San Francisco:
1. Pre-March 2007: Roche, Liberase HI
2. Post-March 2007: Serva/Nordmark GMP NB1 products

Bucher, P., Berney, T. et al. (2005) Transplantation
UCSF - CIT Islet Isolation Protocol

EFFECTIVE ENZYMATIC DISSOCIATION

Donor Selection

Pancreas Perfusion

Enzymatic Pancreas Digestion

Digest Dilution

Purified Islets
Factors that Influence Islet Isolation Outcome

**Donor Selection**
- **Donor Age:** Prefer age <45 years including younger donors
- **Donor Size:** Prefer male pancreas from tall and heavy donors with a large BMI, but not obese.
- Low/No **insulin requirements.**
- Prefer no **family history** of diabetes.

**Pancreas Acceptance & Receipt**
- Procured by **UCSF surgical team** as if for solid organ transplant.
- Maintained in **original cold UW** during trimming (performed in OR)
- Organ cold ischemia time **less than 8 hours.**
Factors That Influence Islet Isolation Outcome

**Pancreas Perfusion**

- Serva Collagenase Reconstitution: >30 minutes at 4°C @16 U/g pancreas.
- Serva Neutral Protease Reconstitution: just prior to perfusion @ 2 U/g pancreas; immediately bring enzyme solution to volume with HBSS 4°C containing 11mM CaCl₂.
- Transfer enzyme solution to cold perfusion trays.
- Perfusion pressures: 4 minutes at 80 psi; 4 minutes at 180 psi.
Factors That Influence Islet Isolation Outcome

**Enzymatic Pancreas Digestion**

- Add 2.5 mL of Pulmozyme (DNase) to Ricordi Chamber.
- Warm enzyme/digestion solution to 37°C with in 4-5 minutes.
- Circulate enzyme solution at approx. 120 mL/minute.
- Digestion End Point: (14-16 minutes)
  1. Islets: > 40 Islets (both free and or embedded).
  2. Acinar: < 100-300 um in size.
Factors that Influence Islet Isolation Outcome

**Digest Dilution**

- Flush chamber with **room temperature** RPMI at 240mL/minute while keeping chamber at 30°C.
- Dilute digest into 1L flask containing RPMI; **5% HSA**; 10,000 U heparin; 100 U Insulin.
- Centrifuge diluted digest immediately (140 x g; 3 minutes)
- Pool digested pancreas into Cold Storage Solution at 4°C containing 10% pentastarch; **1.25% HSA**; 10,000 U heparin; 100 U insulin.

**Islet Purification**

- Optiprep Gradient: Light density 1.06; Heavy density 1.10.
- Load a maximum of 20 mL of digest per Cobe bag.
- Load tissue onto gradient at **20 mL/minute**.
- Stop the loading periodically when tissue is observed forming a thick line in Cobe bag.
### UCSF: Serva/Nordmark Collagenase and Liberase Experience

<table>
<thead>
<tr>
<th><strong>Date of Manufacture</strong></th>
<th>Liberase (n=9)</th>
<th>Serva (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor Pancreas Information</strong></td>
<td>Average</td>
<td>Average</td>
</tr>
<tr>
<td>Preservation Solution</td>
<td>UW</td>
<td>UW</td>
</tr>
<tr>
<td>Donor's age / BMI</td>
<td>45y / 36</td>
<td>32y / 32.6</td>
</tr>
<tr>
<td>Donor Cold Ischemia</td>
<td>6.0 hr</td>
<td>6.5 hr</td>
</tr>
<tr>
<td>Collagenase Units (Wunsch)</td>
<td>2440</td>
<td>1860</td>
</tr>
<tr>
<td>Neutral Protease Units</td>
<td>71,500 (caseinase)</td>
<td>215 (DMC)</td>
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<thead>
<tr>
<th><strong>Digestion Calculations</strong></th>
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<tbody>
<tr>
<td>Collagenase Units &amp; Neutral Protease units /g Trimmed Pancreas</td>
<td>19.9 &amp; 583</td>
</tr>
<tr>
<td>Endotoxin Units/mg</td>
<td>9.7</td>
</tr>
<tr>
<td>Pancreas weight (g)</td>
<td>122.6</td>
</tr>
<tr>
<td>% Digested Tissue</td>
<td>71.5%</td>
</tr>
<tr>
<td>IEQ/g Trimmed Pancreas</td>
<td>5,450</td>
</tr>
<tr>
<td>IEQ/g Digested Tissue</td>
<td>7,460</td>
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<table>
<thead>
<tr>
<th><strong>Functional Data</strong></th>
<th></th>
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<tbody>
<tr>
<td>Glucose Stimulation Index</td>
<td>4.0</td>
</tr>
<tr>
<td>% Viability</td>
<td>99.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Purified IEQ</strong></th>
<th>Liberase: 573,900</th>
<th>Serva: 582,200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplanted IEQ (post-culture)</td>
<td>Liberase: 508,882</td>
<td>Serva: 502,100</td>
</tr>
<tr>
<td>% Recovery after 48hr culture</td>
<td>90%</td>
<td>88%</td>
</tr>
</tbody>
</table>
Conclusion

- Serva GMP Collagenase NB1 is a functional and viable product.
- Allows for younger donor selection.
- Isolation of adequate IEQ necessary for clinical transplant.
- Low organ-to-organ variability with consistent pancreas digestion.

UCSF Experience:
- Serva NB1 Collagenase: 9 of 12 pancreas transplanted.
- Roche Liberase HI: 9 of 19 pancreas transplanted.
Acknowledgements

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