

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

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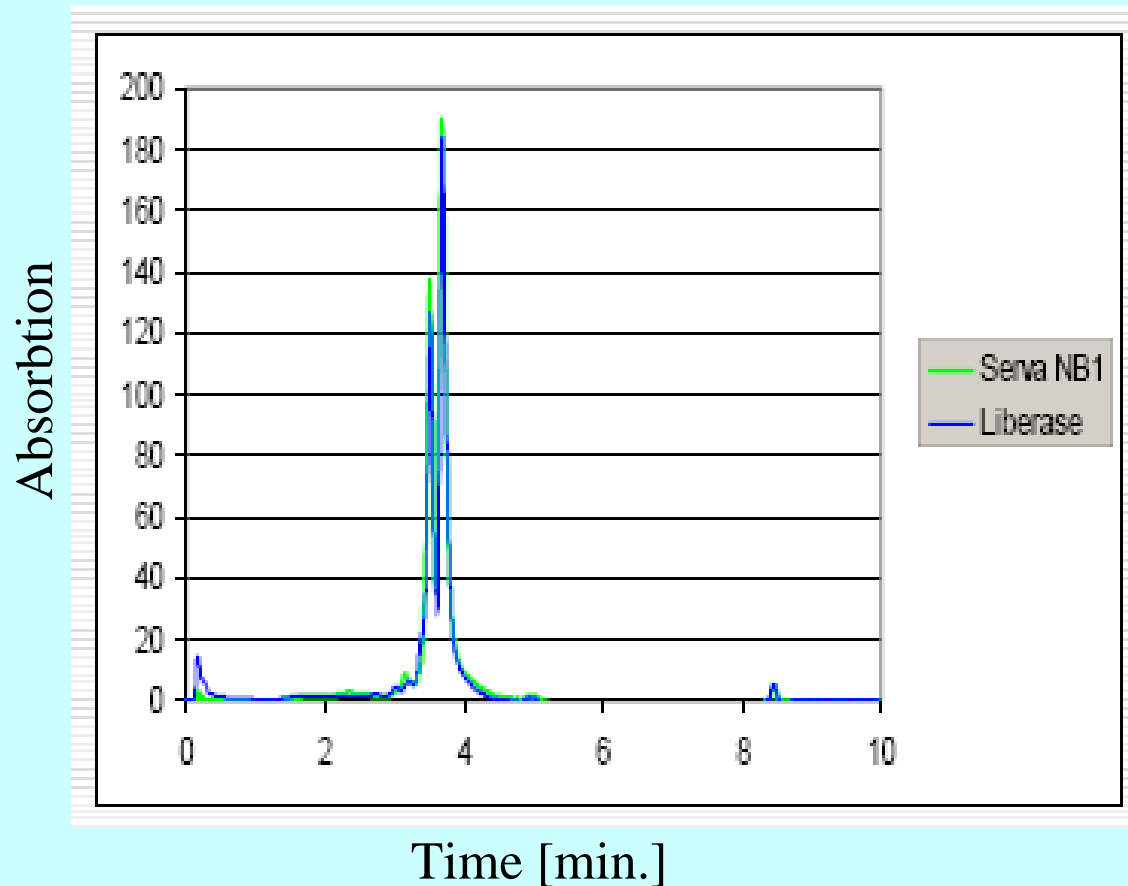
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# Bovine Product-Free Serva NB1 Collagenase and Roche Liberase have a similar HPLC Profile

University of California San Francisco:

1. Pre-March 2007: Roche, Liberase HI
2. Post-March 2007: Serva/Nordmark GMP NB1 products



# UCSF - CIT Islet Isolation Protocol

## EFFECTIVE ENZYMATIC DISSOCIATION

Donor Selection

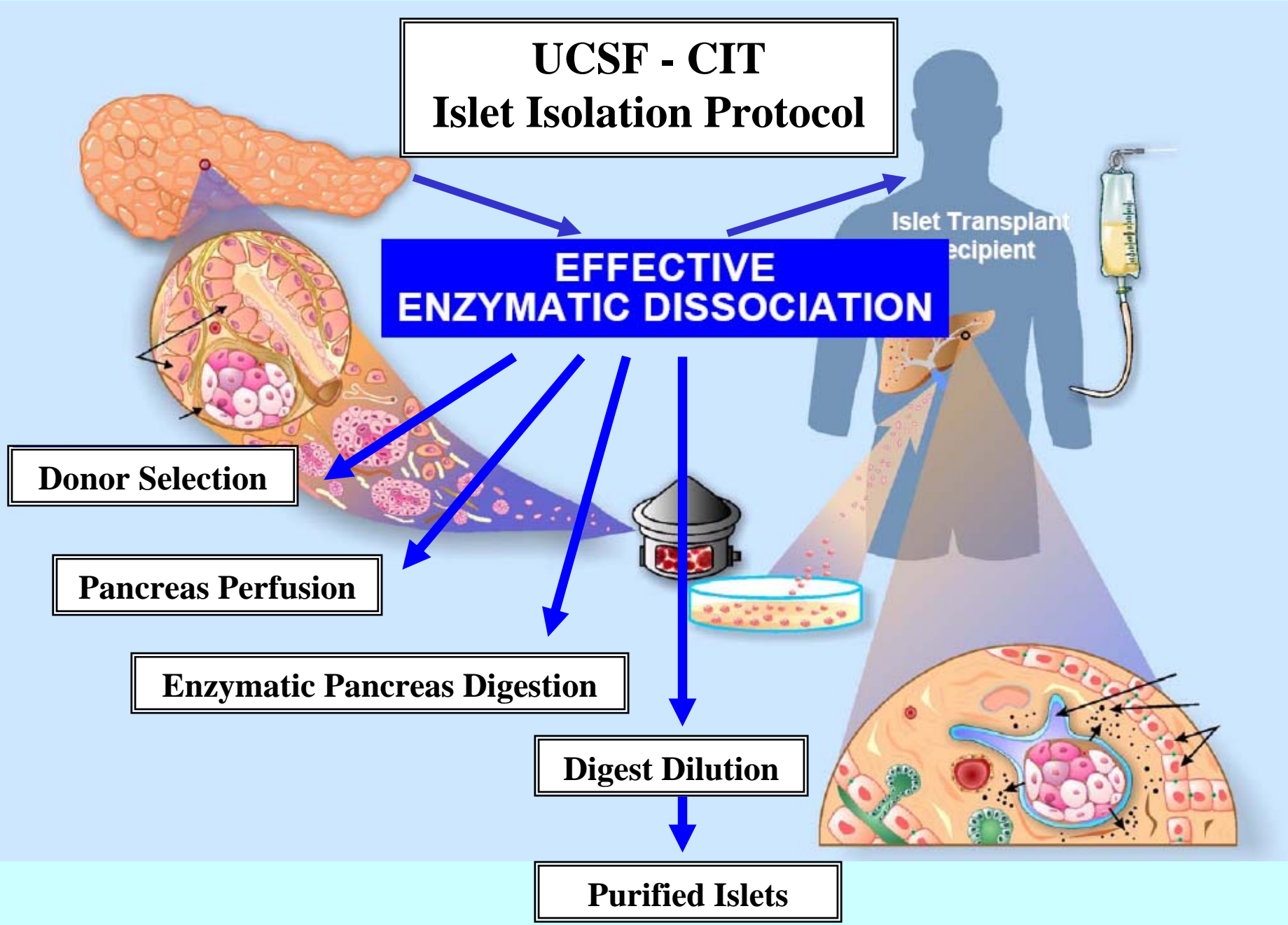
Pancreas Perfusion

Enzymatic Pancreas Digestion

Digest Dilution

Purified Islets

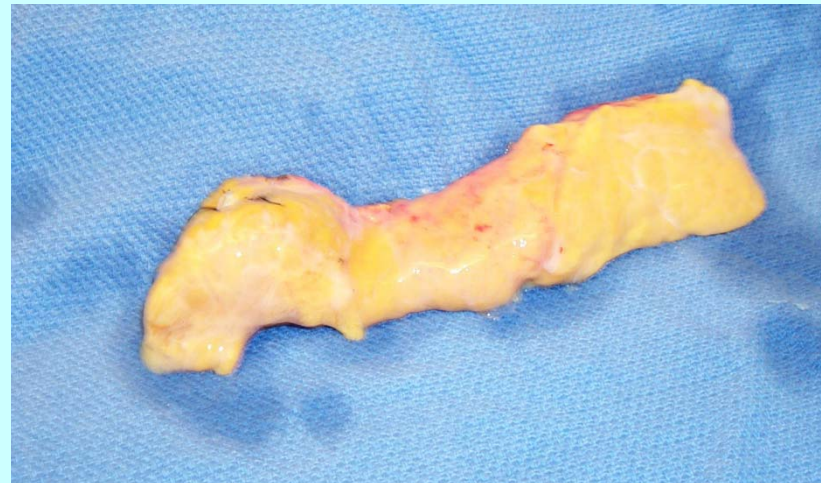
Islet Transplant  
Recipient



# 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.



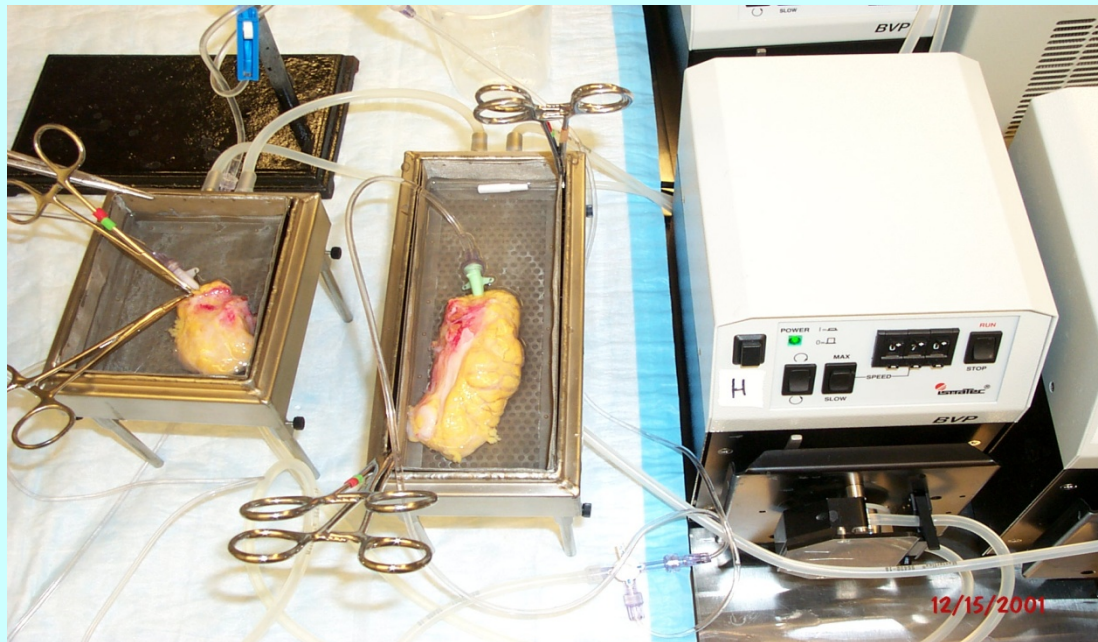
## Pancreas Acceptance & Receipt

- 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

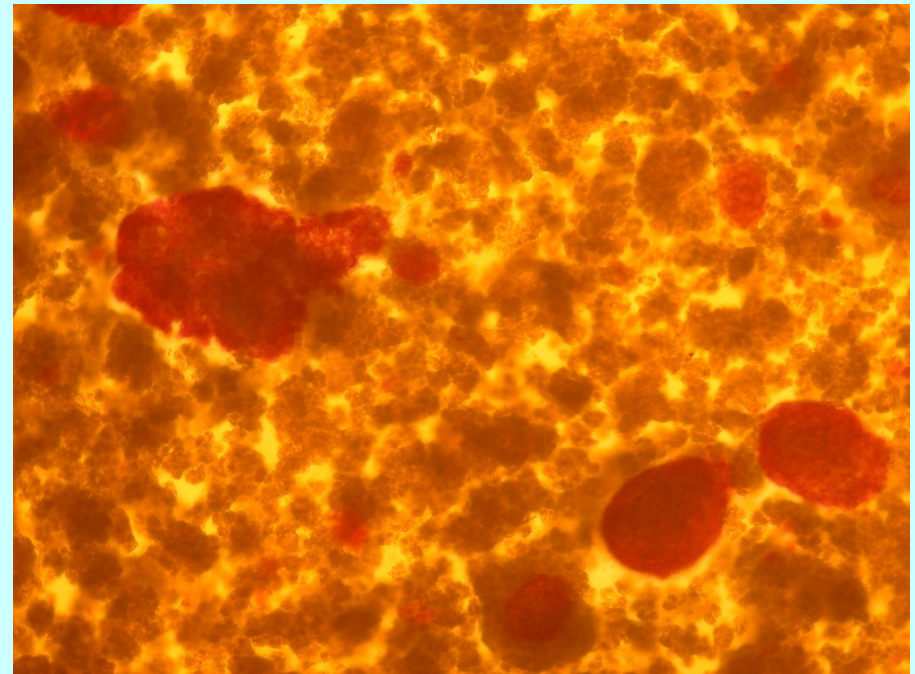
- Serva Collagenase Reconstitution: >30 minutes at 4°C @ 16 U/g pancreas.
- Serva Neutral Protease Reconstitution: **just prior to perfusion** @ 2 U/g pancreas; immediately bring enzyme solution to volume with HBSS 4°C containing **11mM CaCl<sub>2</sub>**.
- Transfer enzyme solution to **cold perfusion 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** within 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

<b>Date of Manufacture</b>	<b>Liberase (n=9)</b>		<b>Serva (n=9)</b>
<b>Donor Pancreas Information</b>	<b>Average</b>		<b>Average</b>
Preservation Solution	UW		UW
Donor's age / BMI	45y / 36		32y / 32.6
Donor Cold Ischemia	6.0 hr		6.5 hr
Collagenase Units (Wunsch)	2440		1860
Neutral Protease Units	71,500 (caseinase)		215 (DMC)
<b>Digestion Calculations</b>			
Collagenase Units & Neutral Protease units /g Trimmed Pancreas	19.9 & 583		18.4 & 2.12
Endotoxin Units/mg	9.7		0.71
Pancreas weight (g)	122.6		101.4
% Digested Tissue	71.5%		68.0%
IEQ/g Trimmed Pancreas	5,450		7,850
IEQ/g Digested Tissue	7,460		11,300
<b>Functional Data</b>			
Glucose Stimulation Index	4.0		3.19
% Viability	99.5		98.3
Purified IEQ	573,900		582,200
Transplanted IEQ (post-culture)	508,882		502,100
% Recovery after 48hr culture	90%		88%



# 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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