Investigating the Effective Use and Standardization of Roche Liberase™ Collagenase Blends

ICR Program Islet Workshop

July 11 2005

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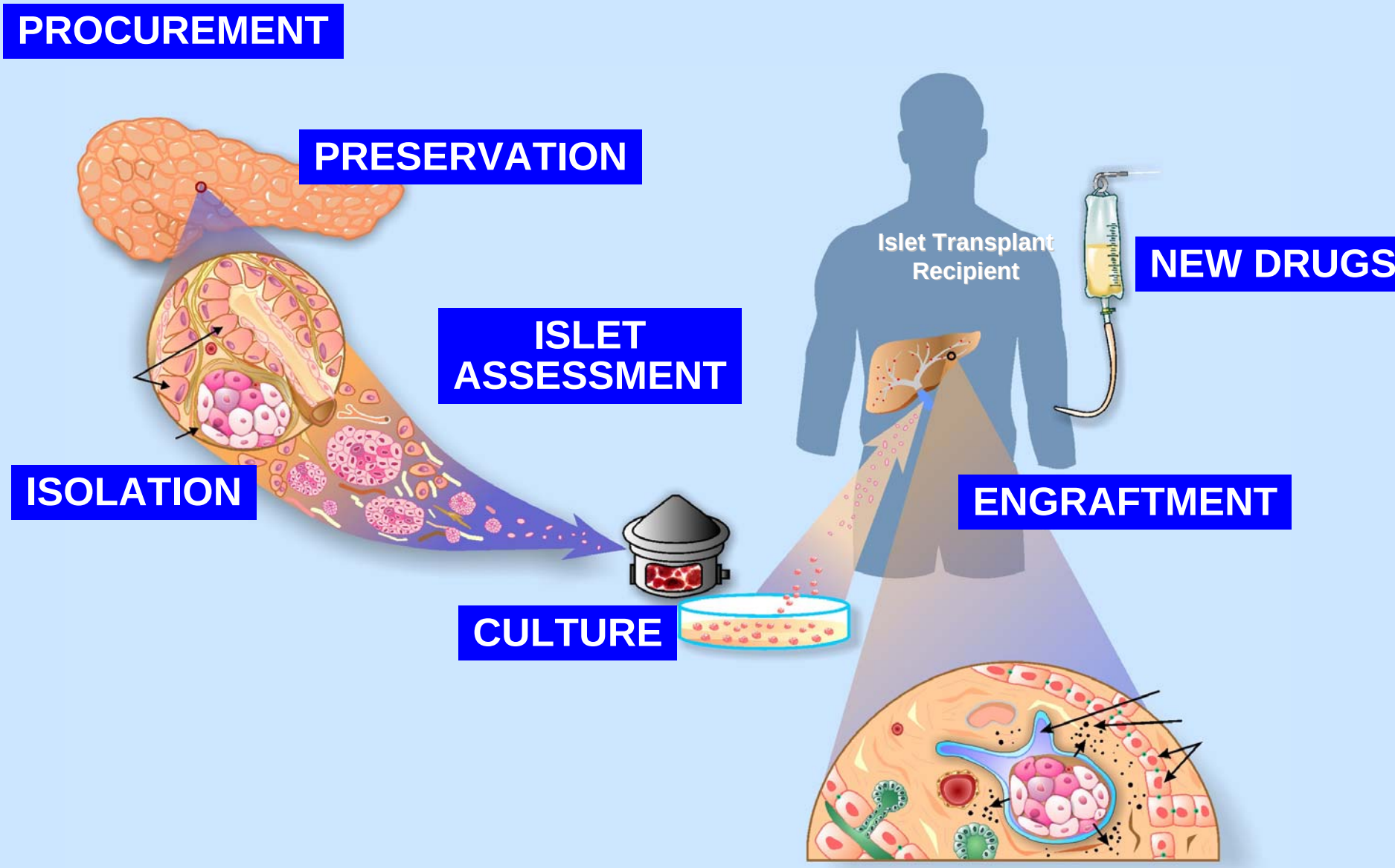
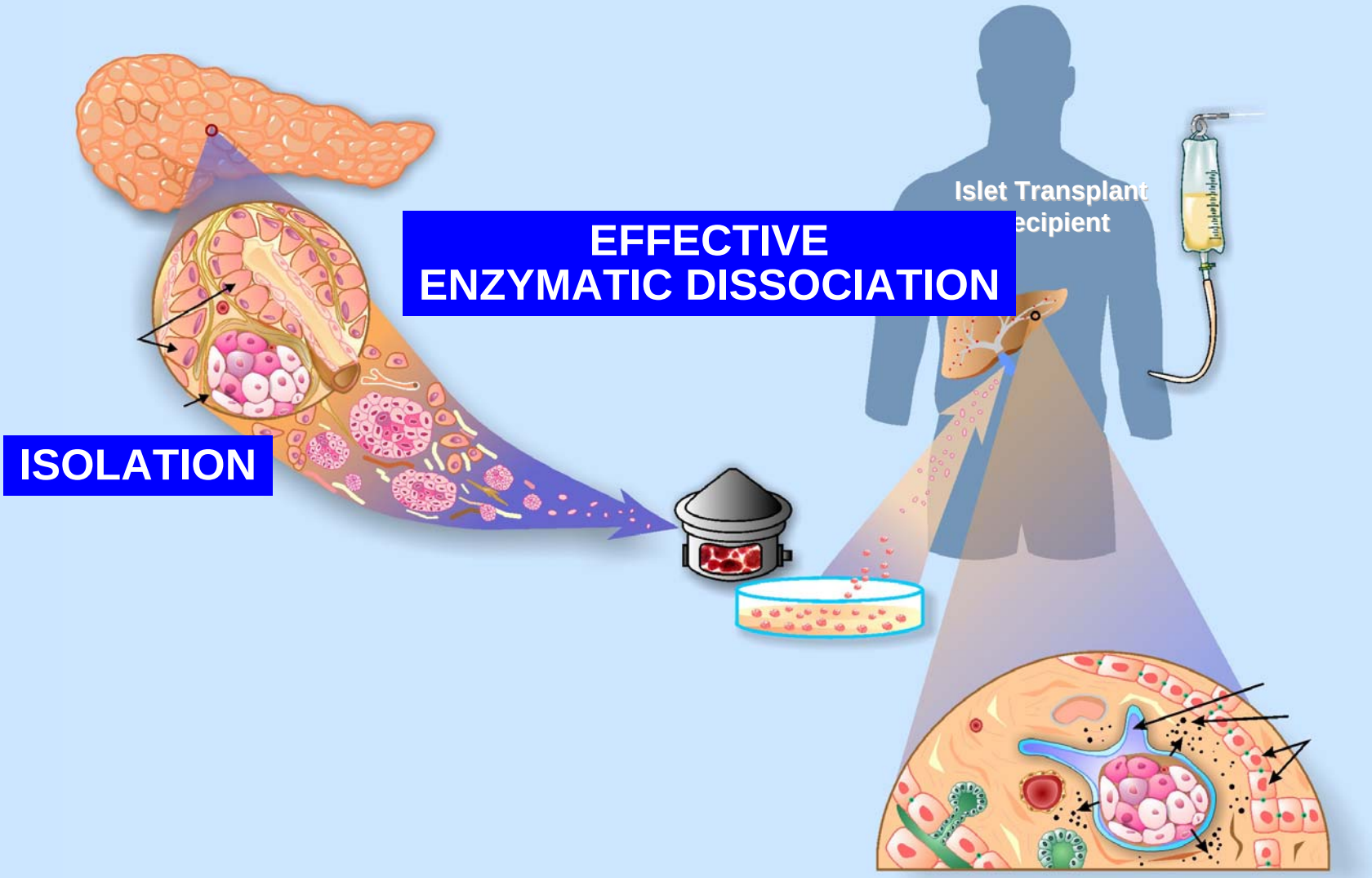
Edmonton CANADA

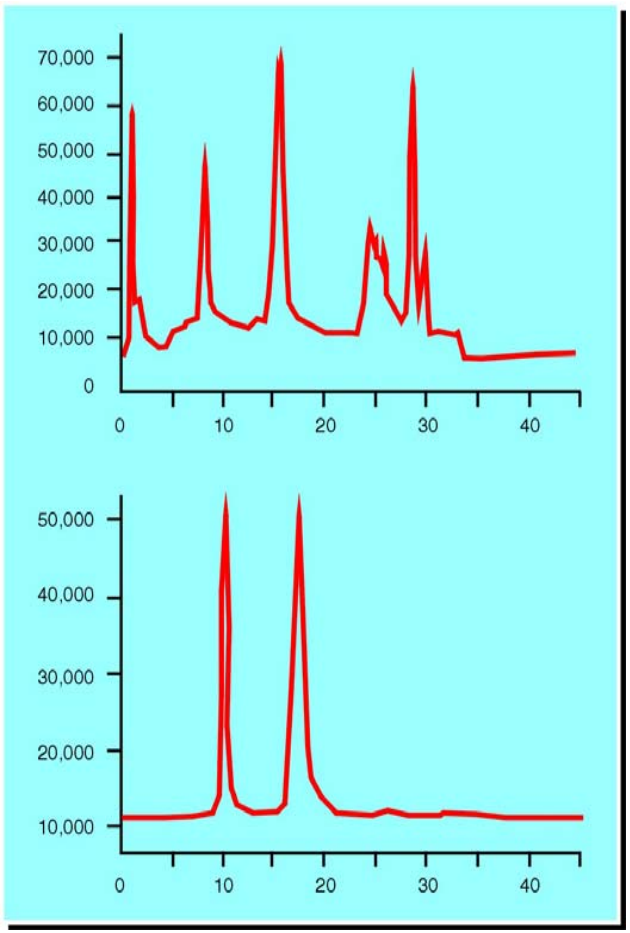
Challenges and Emerging Opportunities

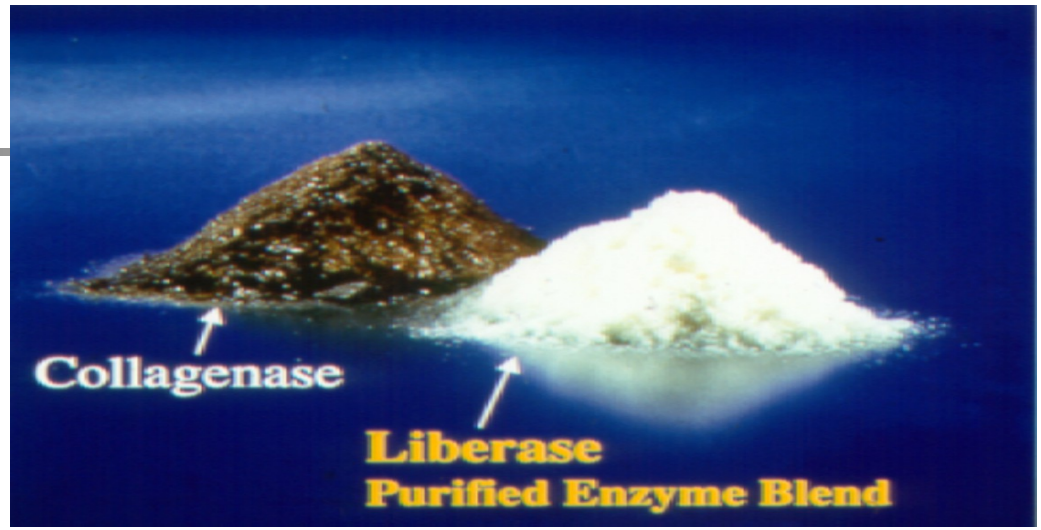


Challenges and Emerging Opportunities



Development of Liberase Enzyme 1995





Initial Goal of Liberase Development

- Lot to lot consistency
- Greater stability
- Keeping endotoxin low
- Free from contamination
- Tissue specific

Overview

- Clinical Use of Liberase™ Blends
- Origins of Liberase
- Enzyme Variability and Stability

Section I – Clinical use of Liberase™

Collagenase Blends in Islet Transplantation

Digestion Process

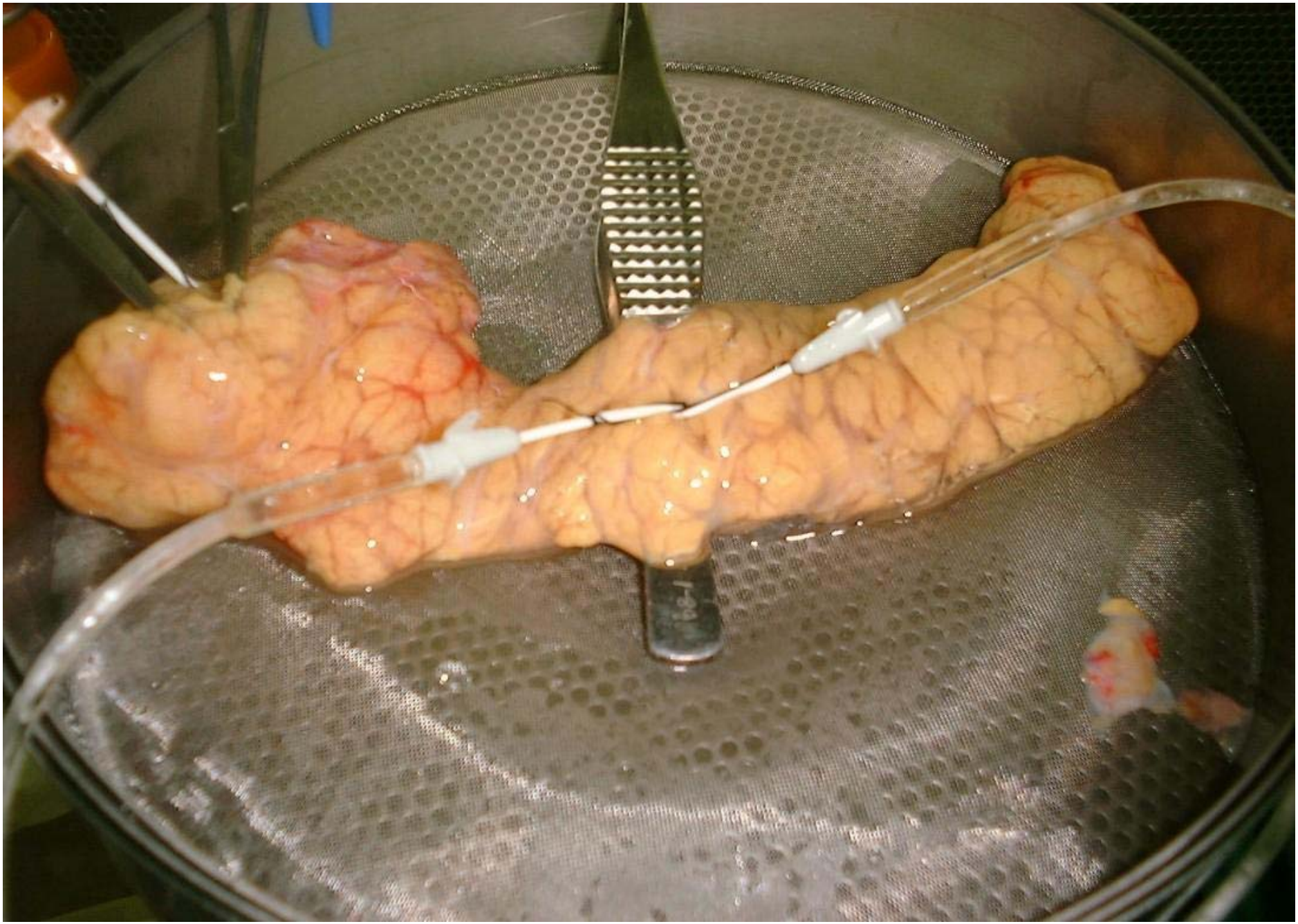
1. **Physical Distention of the pancreas**
2. **Enzymatic Dissociation**
3. **Dilution (Cessation of enzyme activity)**

Liberase™ Reconstitution

- Vial stored at –80C equilibrated to ambient temperature for 5-10 minutes
- Surface disinfection with 70% EtOH and aseptic cap removal
- Reconstitute vial with 40mL of cold, sterile Perfusion Solution (HBSS) for 30 min on ice.
- Reconstitute to a final volume of 350 mL cold, sterile Perfusion Solution

Pancreas Perfusion

- Ductal cannulation
- Controlled Distention – 5 min at 80 psi, followed by 5 min at 180 psi, monitor flow based on pressure
- Determine enzyme recovery
- Final dissection to 7-9 pieces, to dissociation chamber (Ricordi).

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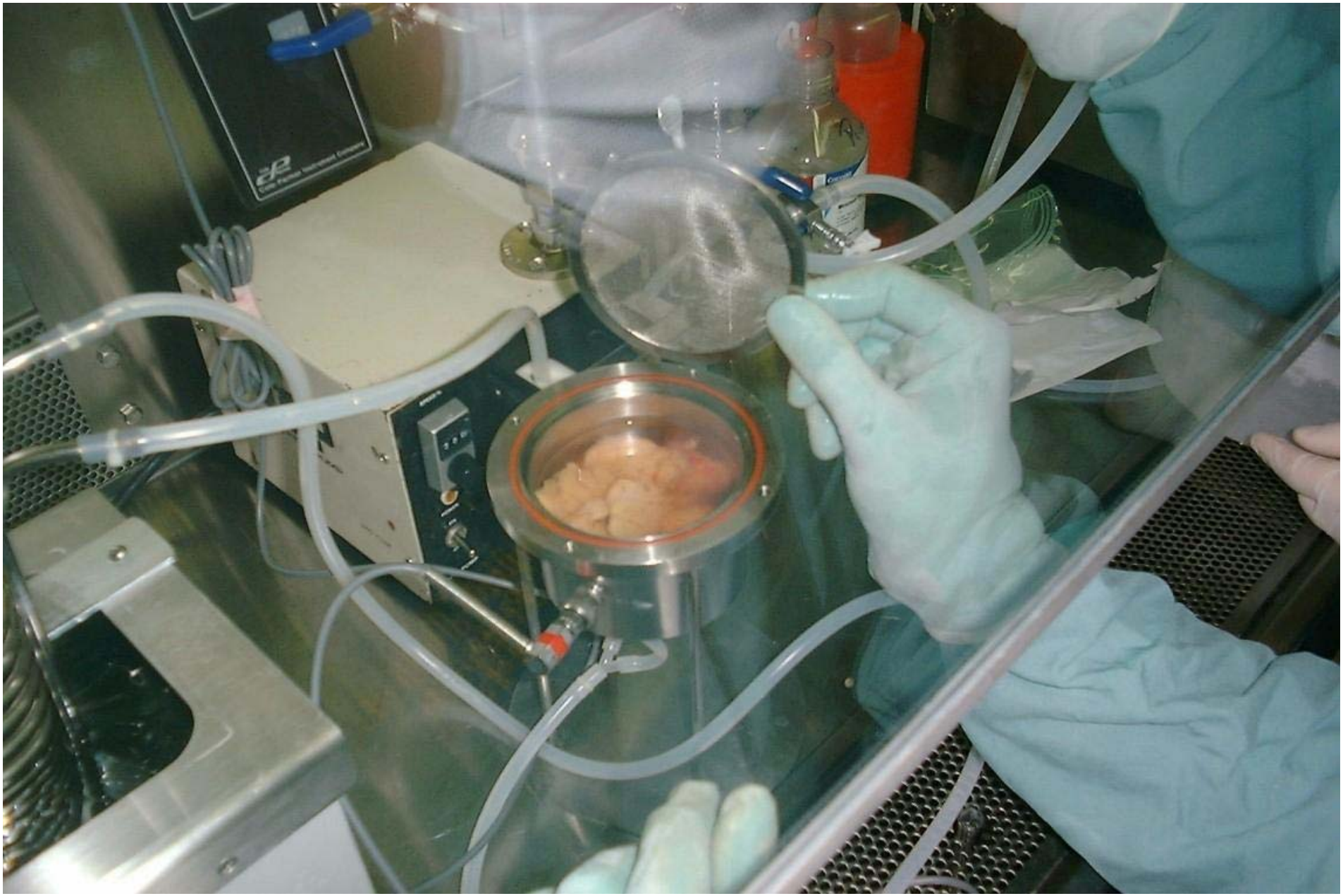
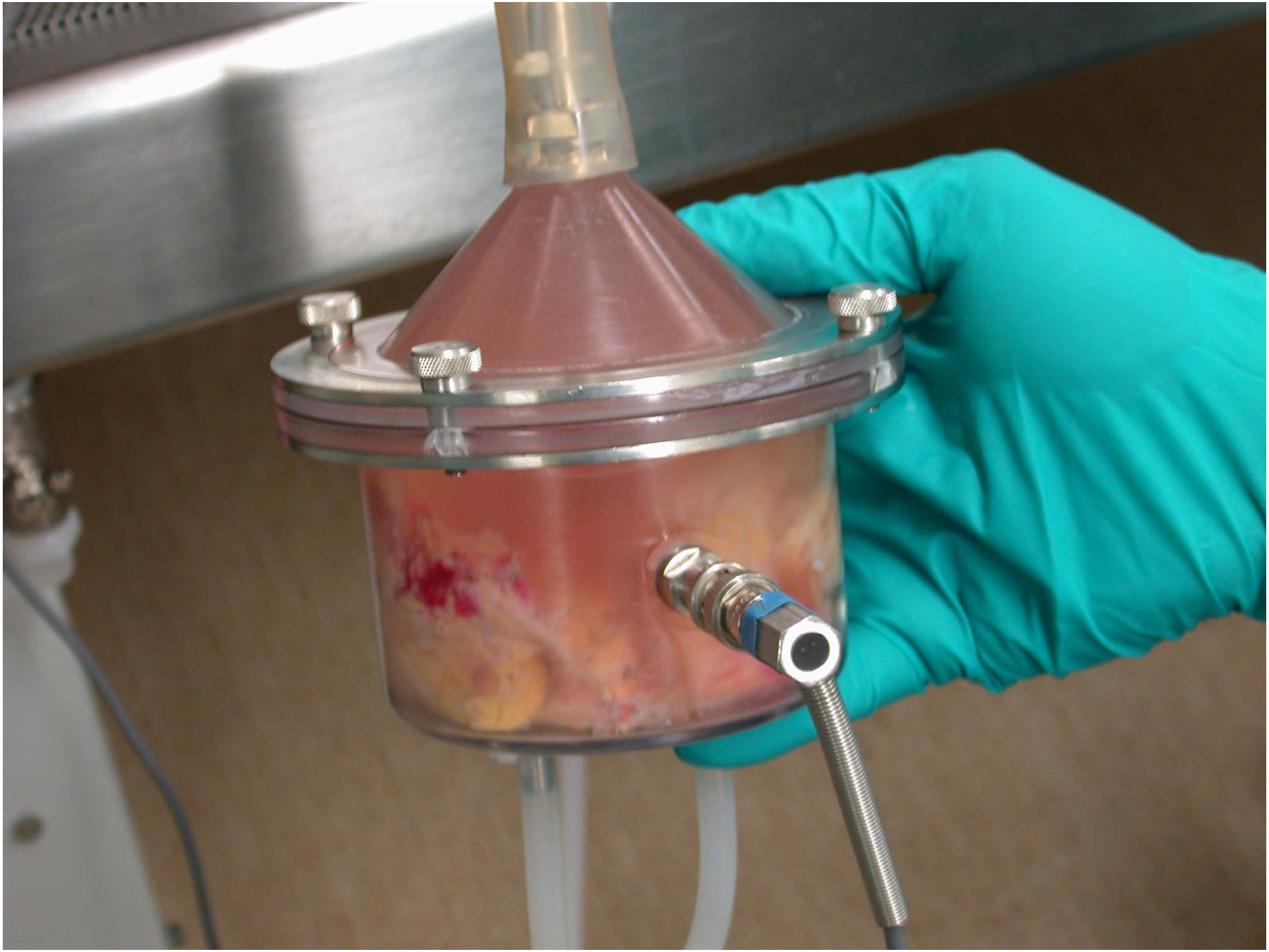
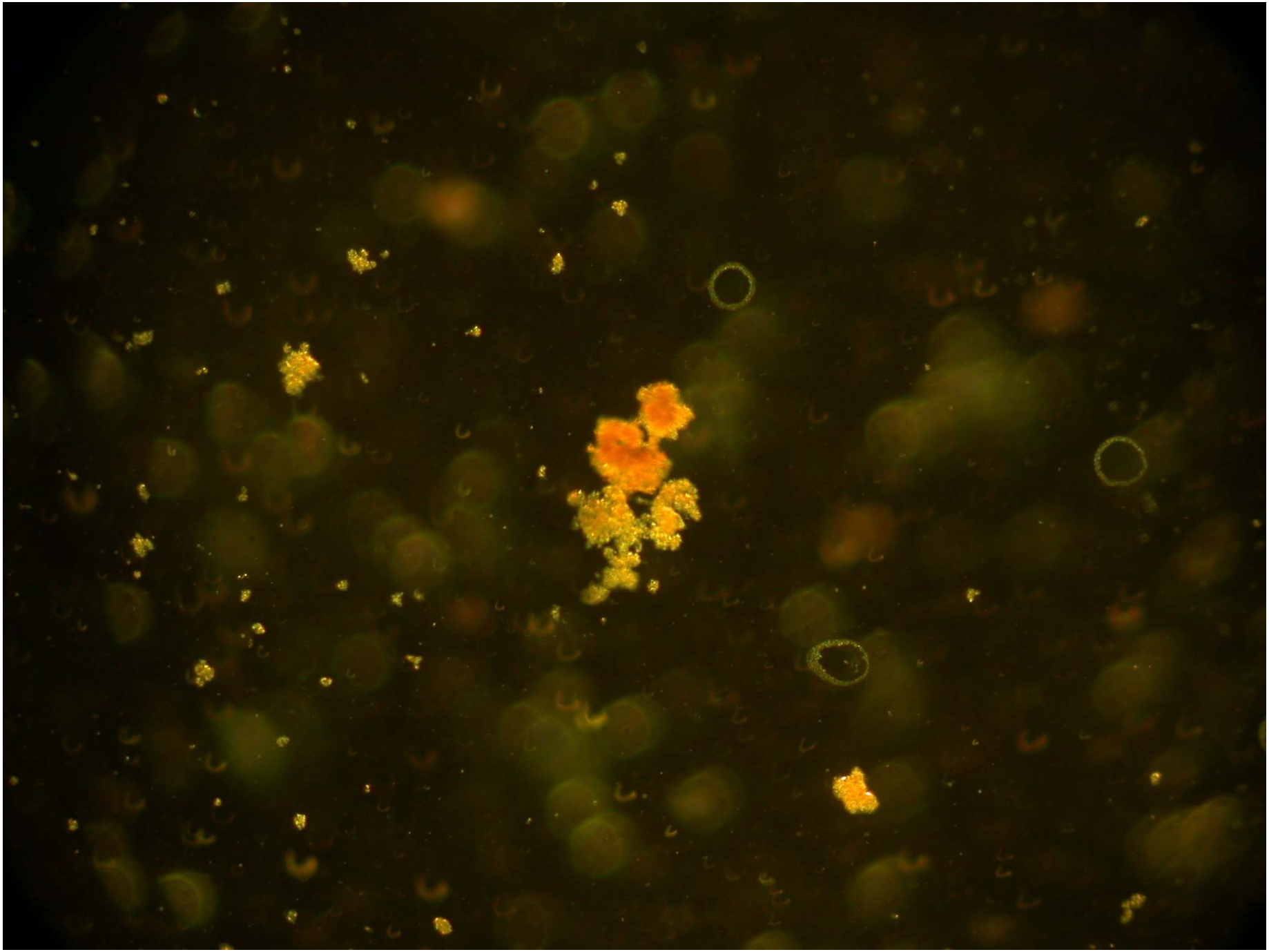
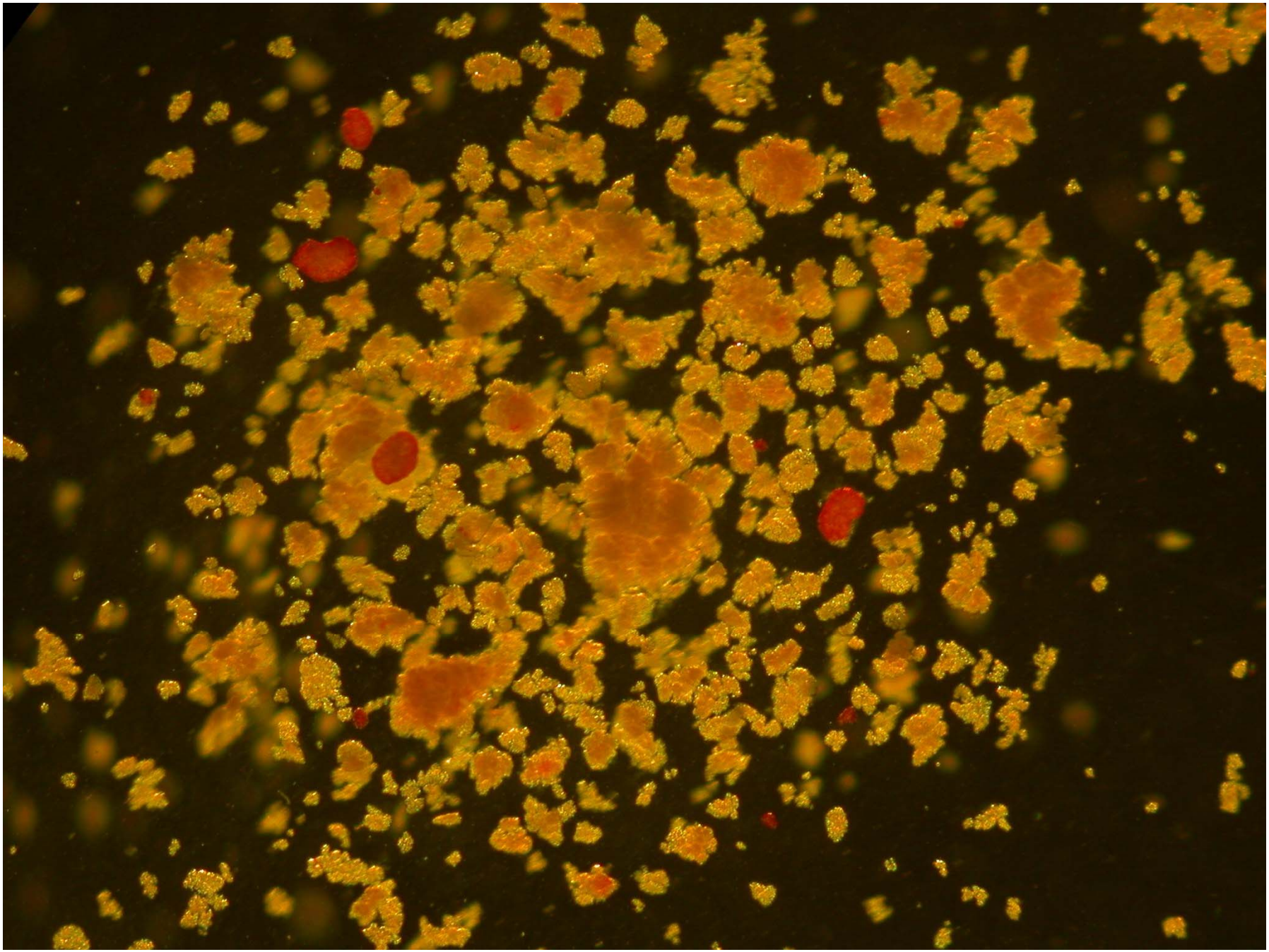
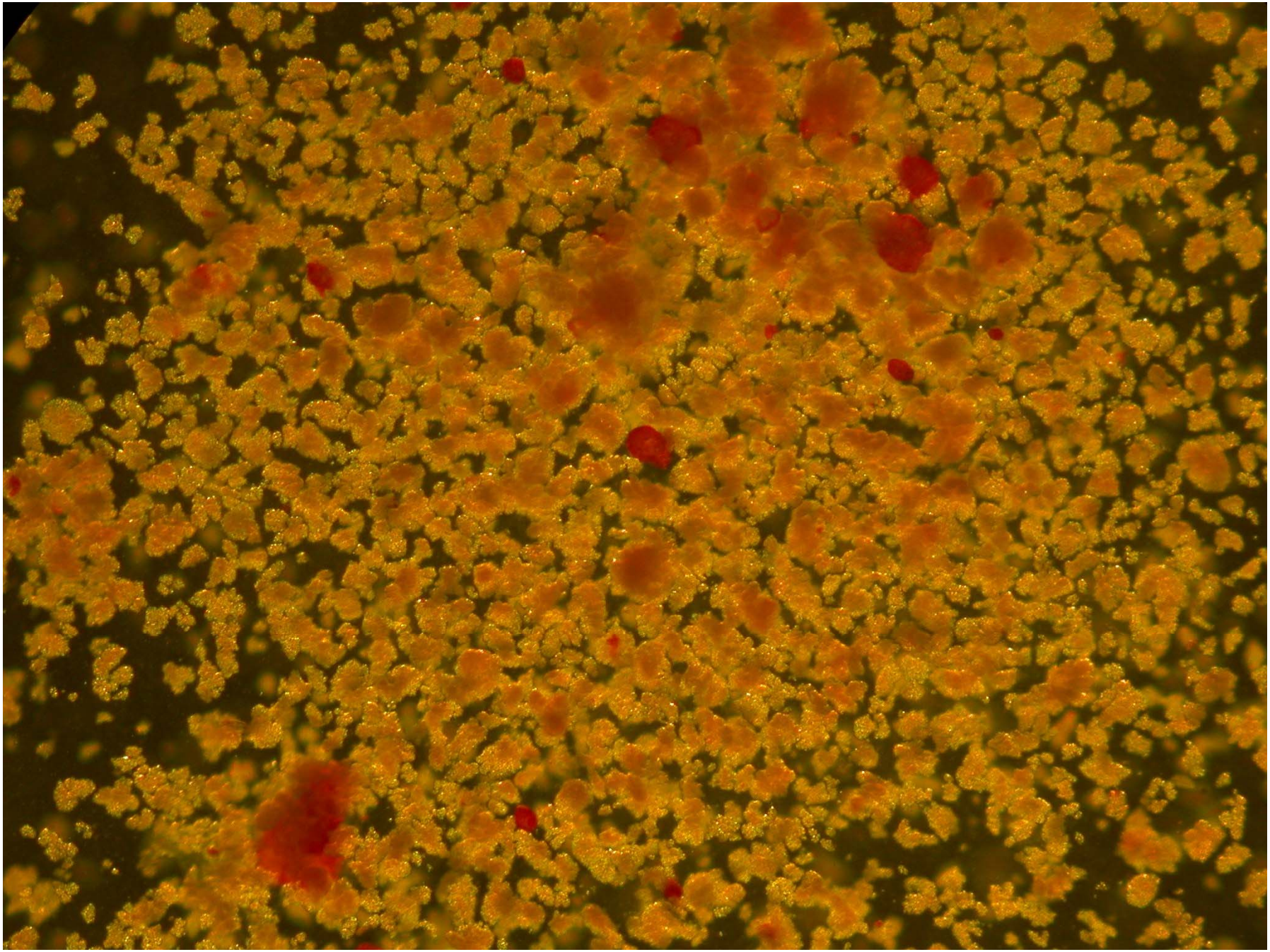
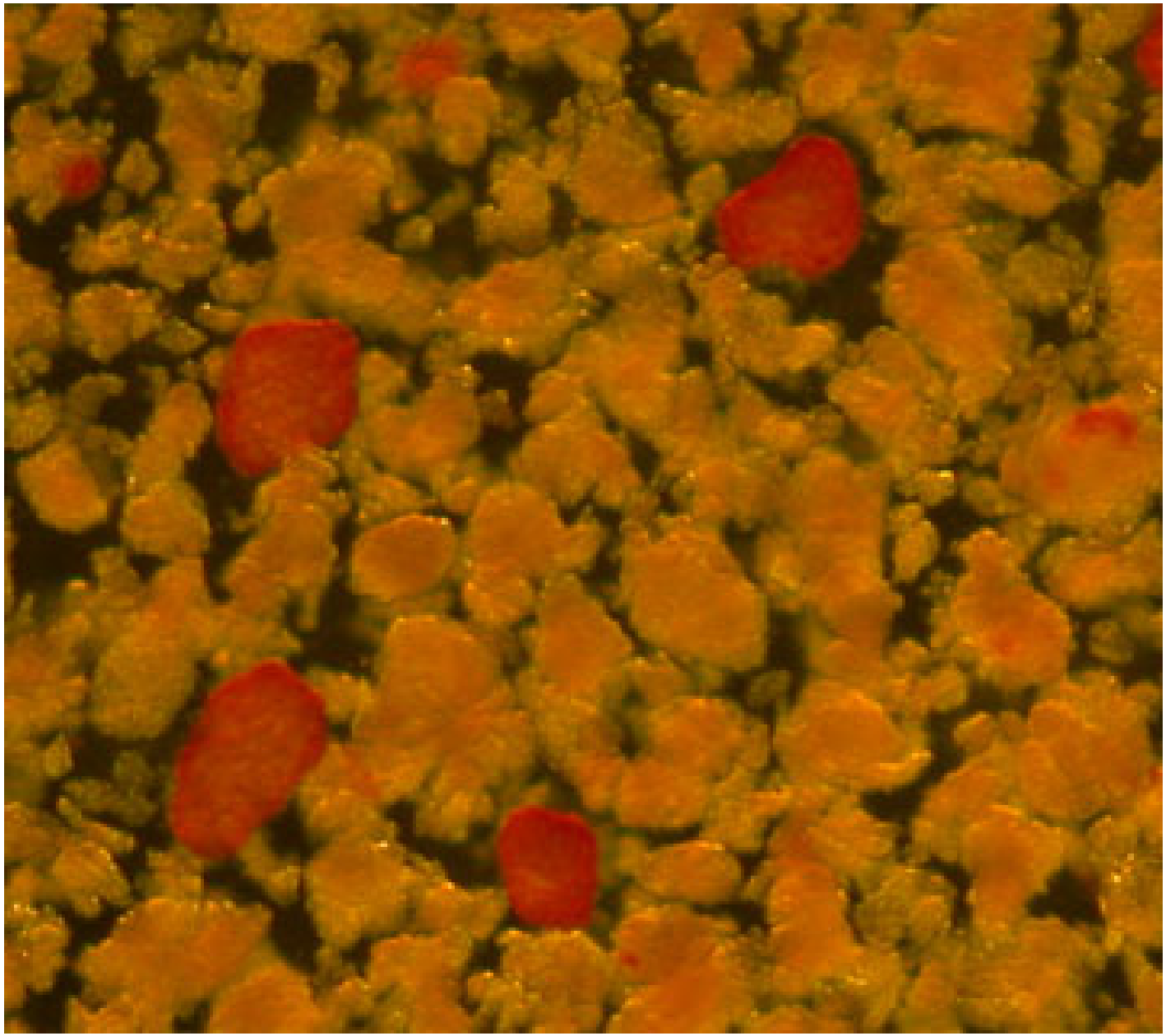












Section II – Origins of Liberase™

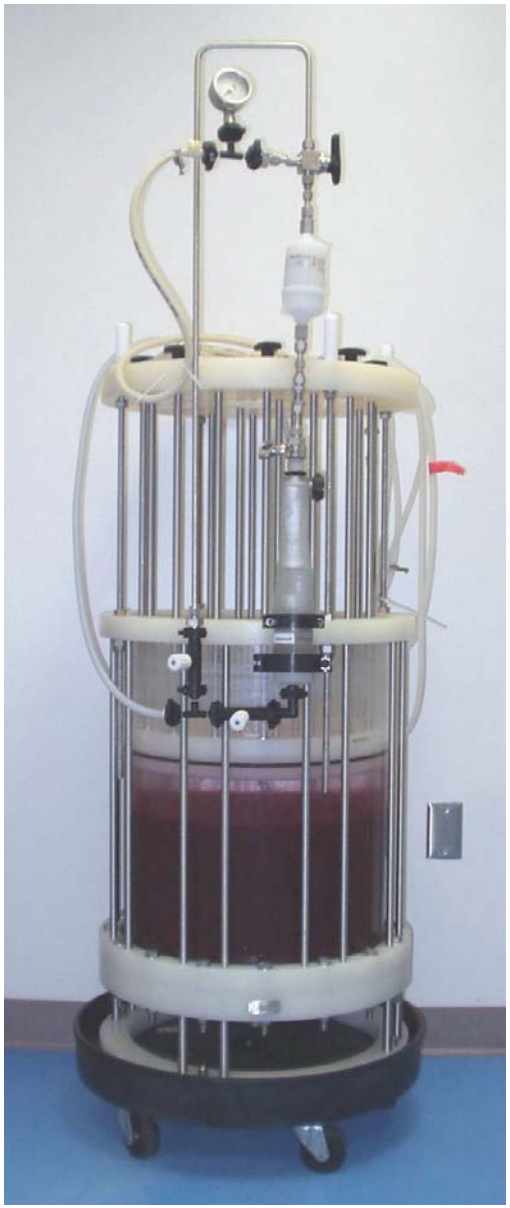
What is Liberase™ HI ?

- Highly purified, lyophilized mixture of collagenases class I and II from *Clostridium histolyticum.* These **isoforms** are synergistic in degrading collagen to cleave islets selectively from the surrounding pancreas.

- The blend also contains a neutral protease (Thermolysin), which dramatically reduces the digestion time by degrading proteins during digestion.

- The purification is intended to remove protease contaminants, endotoxins, and standardize potency.

Liberase Enzyme Manufacture



Dye Affinity Chromatography

- Dilute 50 Liters crude to 400 liters
- Unwanted material flows through
  - neutral protease
  - pigment, debris
  - endotoxin
- Diafilter 200 L to 20 L
- Dilute 20 L to 80 L
- Store overnight at 4ºC

Photo Courtesy of Roche Applied Sciences

Liberase Enzyme Manufacture

Cation Exchange Chromatography

- Diafilter 80 L to 20 L
- Remove clostripain
- Diafilter 100 L to 10 L
- Dilute 10 L to 80 L
- Store overnight at 4ºC

Photo Courtesy of Roche Applied Sciences

Liberase Enzyme Manufacture

Anion Exchange Chromatography

- Diafilter 80 L to 15 L
- Separate collagenase I and II isoforms
- Remove endotoxin
- Diafilter 60 L CII to 2 L
- Diafilter 100 L CI to 2 L
- Store at -20ºC 6-12 months

Photo Courtesy of Roche Applied Sciences

Liberase Enzyme Manufacture

Lyophilization

- -35ºC freeze 2h @ 1 atm
- -35ºC 40-50h under vacuum
- Dry at -10ºC 40-72h
- Dry at 35ºC 24h

Photo Courtesy of Roche Applied Sciences

Liberase manufacture Summary

- Fermentation - 5 days
- Purification - 6 days
  - **5-7 runs over 3 columns**
  - **6000 L buffer**
- Testing - 2 days
- Blending - 1 day
- Lyophilization - 6 days
- Testing - 2-3 days
- Inspection - 1 day

Approx. 3 weeks

Section III
Enzyme Variability and Stability

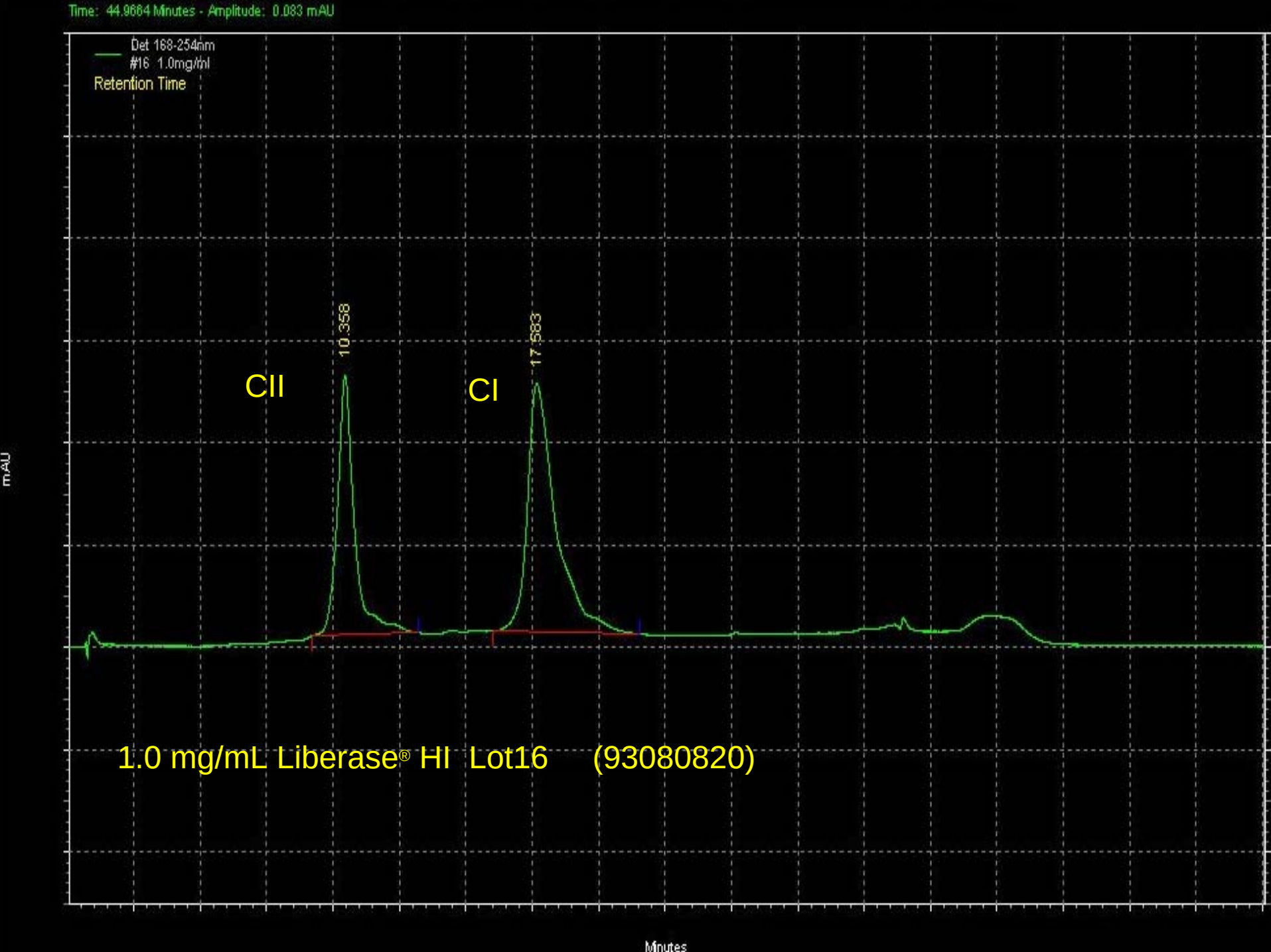
Lot Variability Assessment

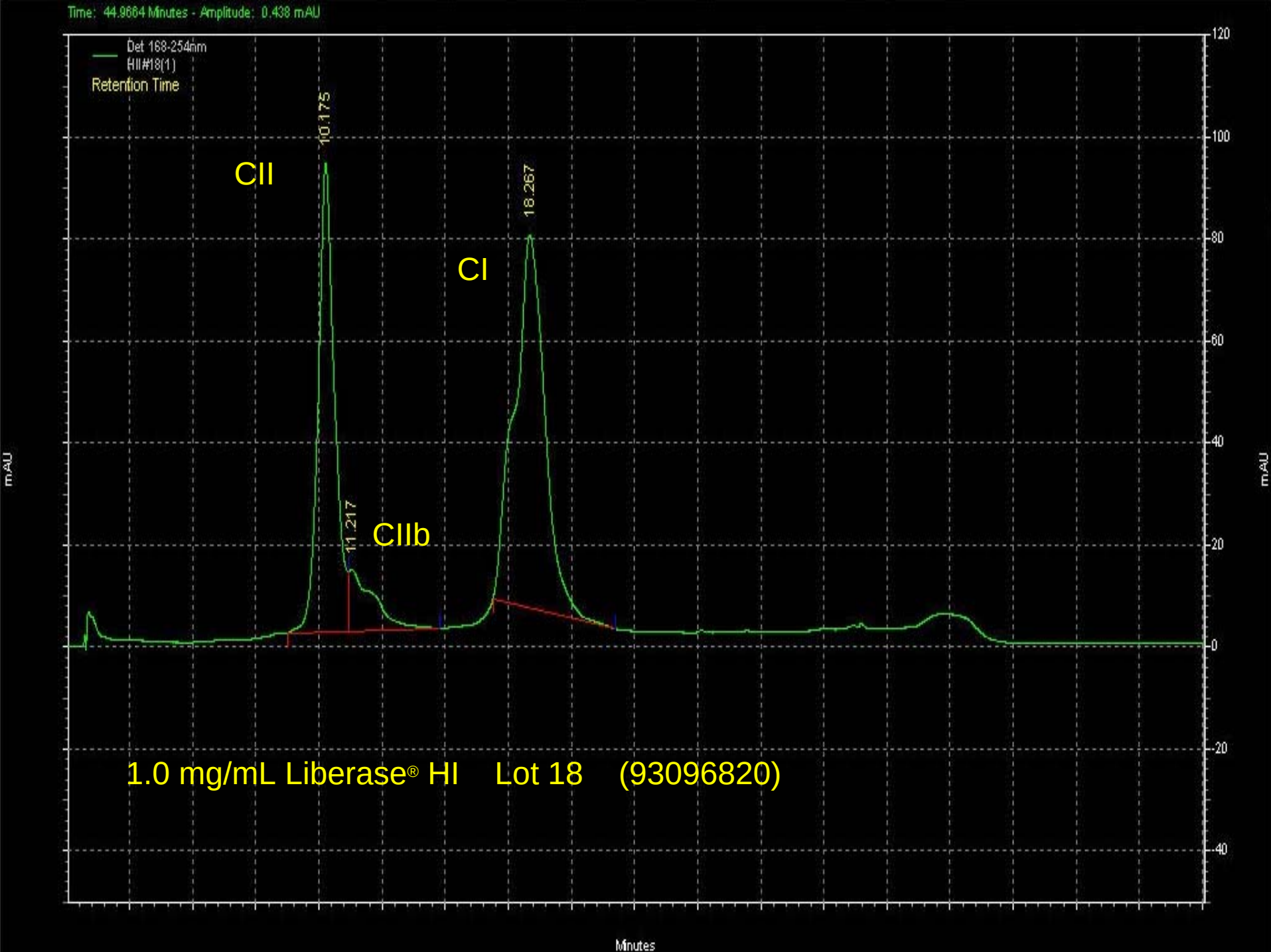
Methodologies:

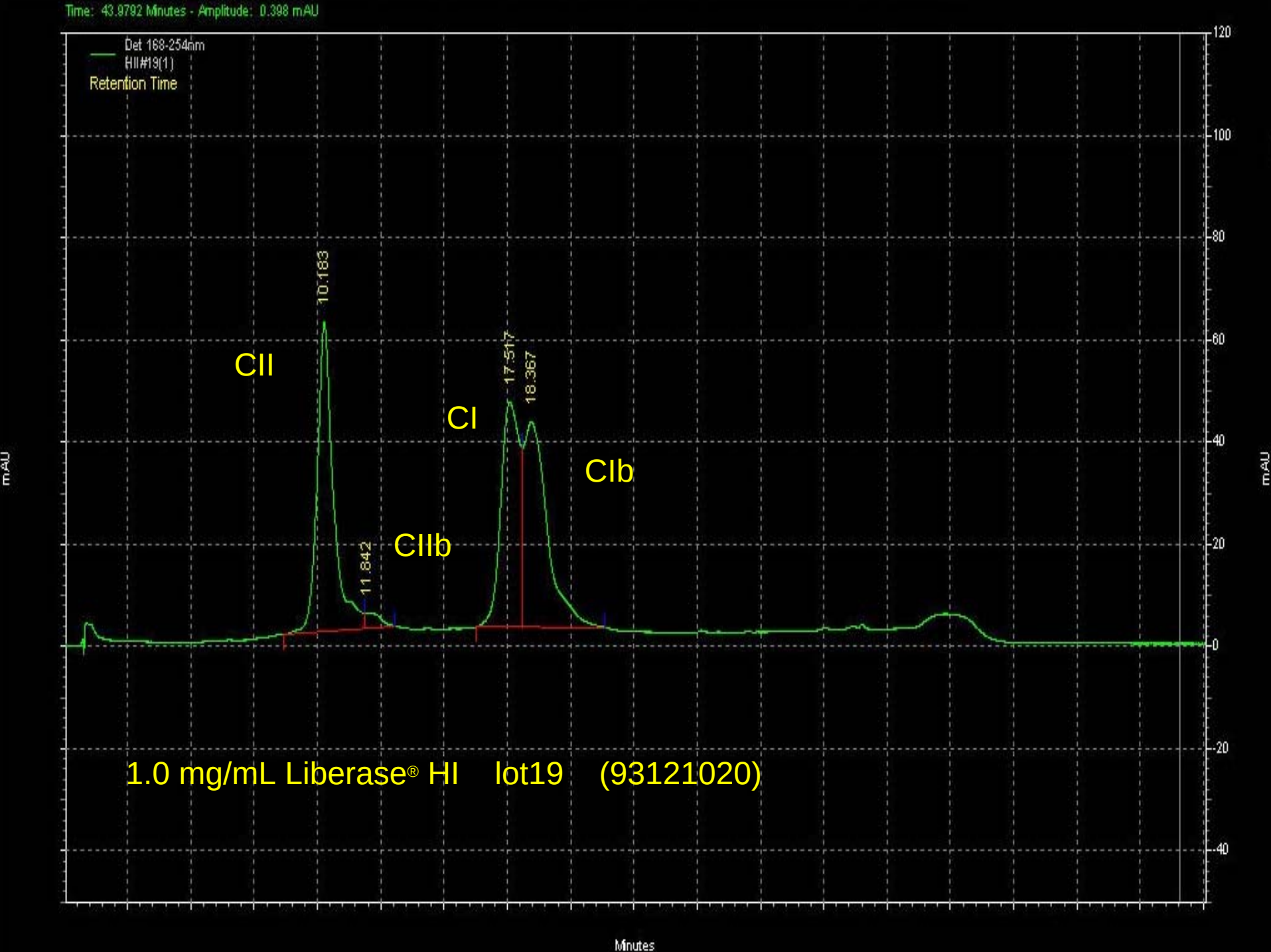
- High Performance Liquid Chromatography (HPLC)
- Kinetic activity analysis (Collagenase and Protease activity)
- Total Protein measurement
- LAL Endotoxin Measurement
- In-process sterility testing

Human Islet Investigations Laboratory


LIQUID HEAD
PRIME PUMP
PRIME LINES
SOLVENT SELECTOR B
1 2 3 4
CHK COM CPU PS
SOLVENT SELECTOR A
1 2 3 4
126 Solvent
LOAD
INJECT
LAMP
CHK
COM
CPU
PS
POWER


Time: 44.9684 Minutes - Amplitude: 0.083 mAU
Det 168-254nm
#16 1.0mg/ml
Retention Time
10.358
17.583
CII
CI
mAU
1.0 mg/mL Liberase® HI Lot16 (93080820)
Minutes


Time: 44.9664 Minutes - Amplitude: 0.438 mAU
Det 168-254nm
HII#18(1)
Retention Time
10.175
18.267
11.217
CII
CI
CIIb
1.0 mg/mL Liberase® HI Lot 18 (93096820)
mAU
Minutes
120
100
80
60
40
20
0
-20
-40


Time: 43.9792 Minutes - Amplitude: 0.398 mAU
Det 168-254nm
HII#19(1)
Retention Time
10.183
11.842
17.517
18.367
CII
CIIb
CI
CIb
1.0 mg/mL Liberase® HI lot19 (93121020)
mAU
Minutes

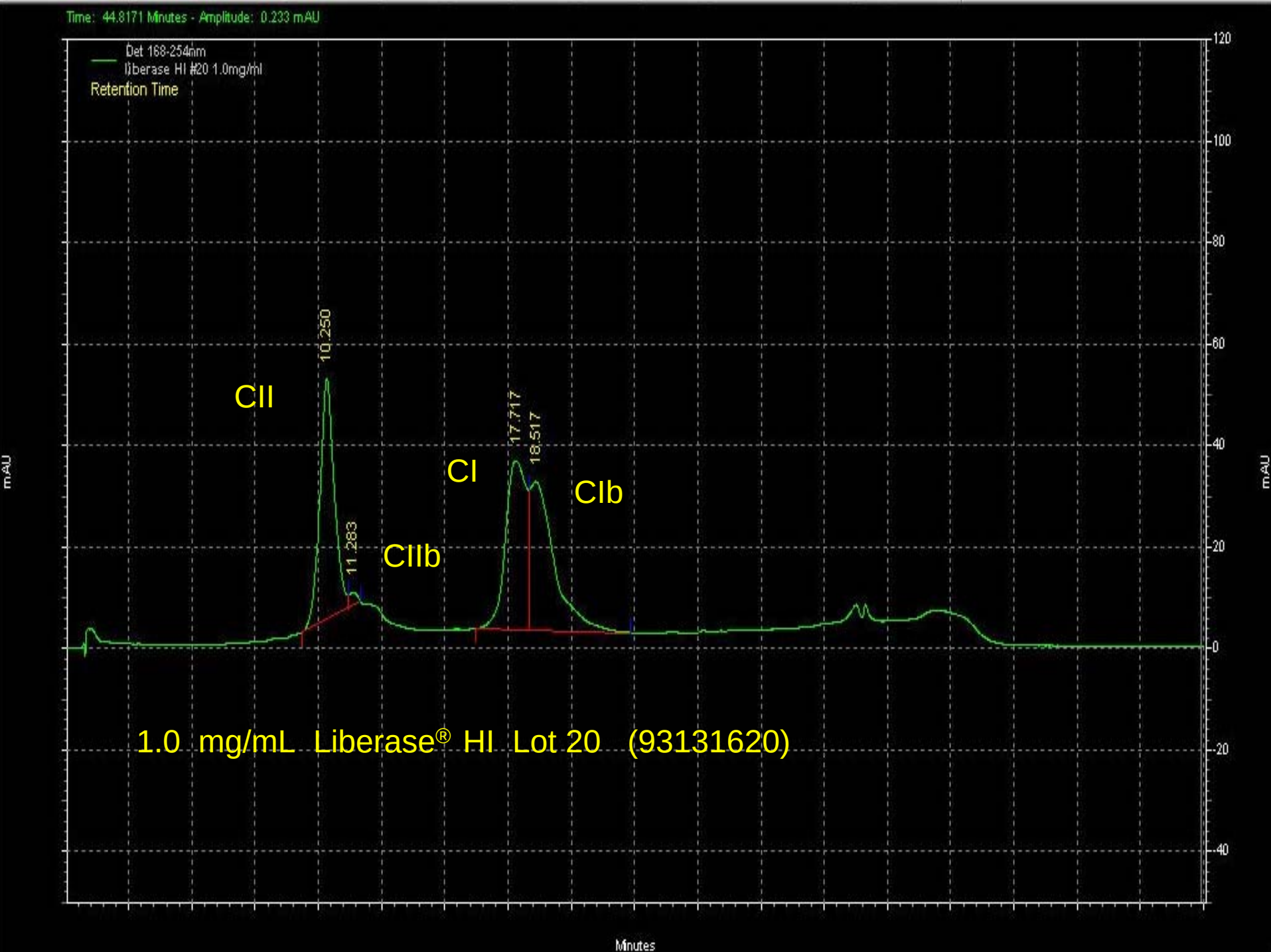
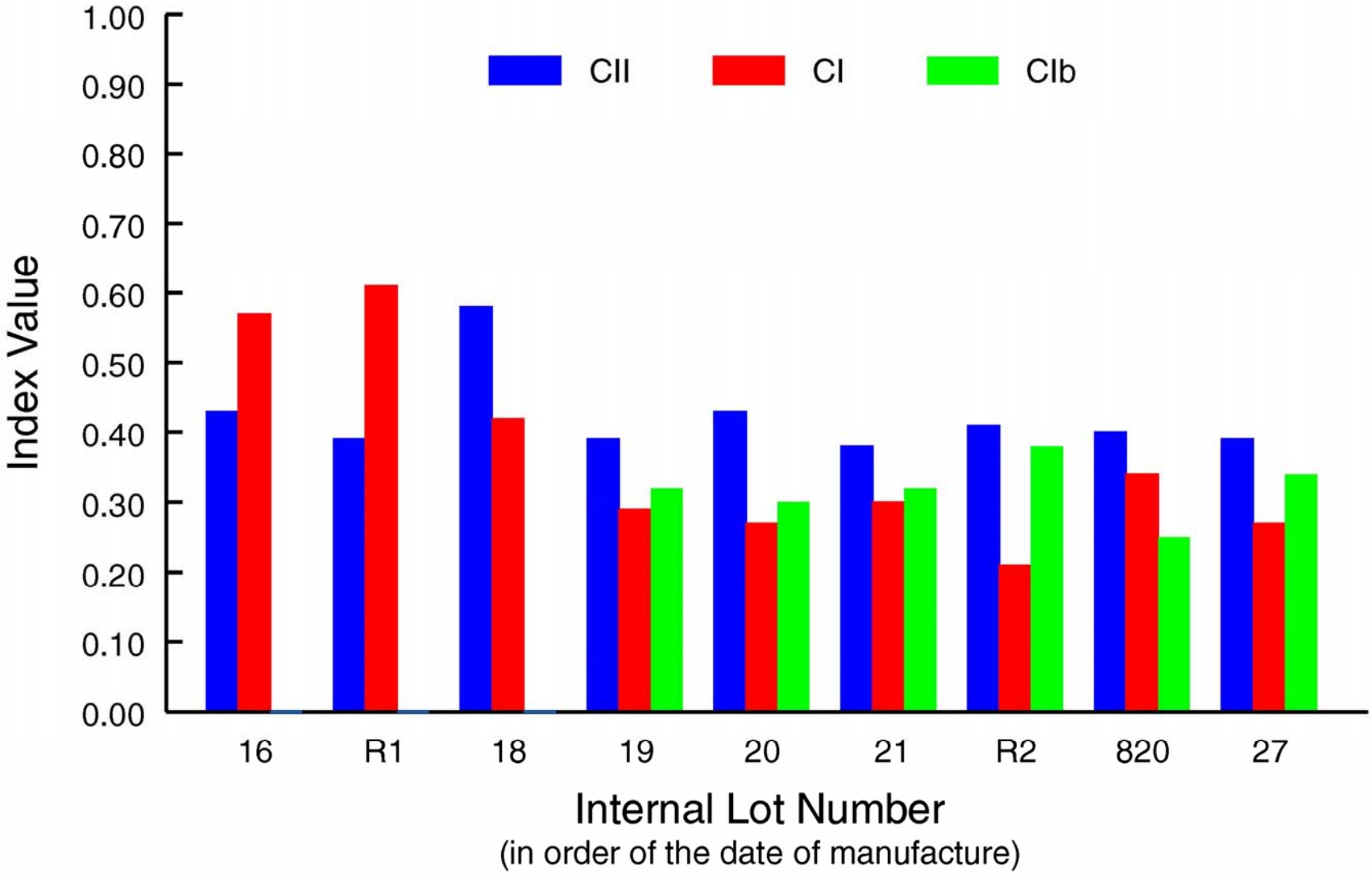

Time: 44.8171 Minutes - Amplitude: 0.233 mAU
Det 168-254nm
Liberase HI #20 1.0mg/ml
Retention Time
mAU
120
100
80
60
40
20
0
-20
-40
10.250
11.283
17.717
18.517
CII
CIIb
CI
CIb
1.0 mg/mL Liberase® HI Lot 20 (93131620)
Minutes

Figure 2

Variation in Liberase™HI Formulation Based on Collagenase Fractionation



Specific Activity of Fractioned CI vs. CIb

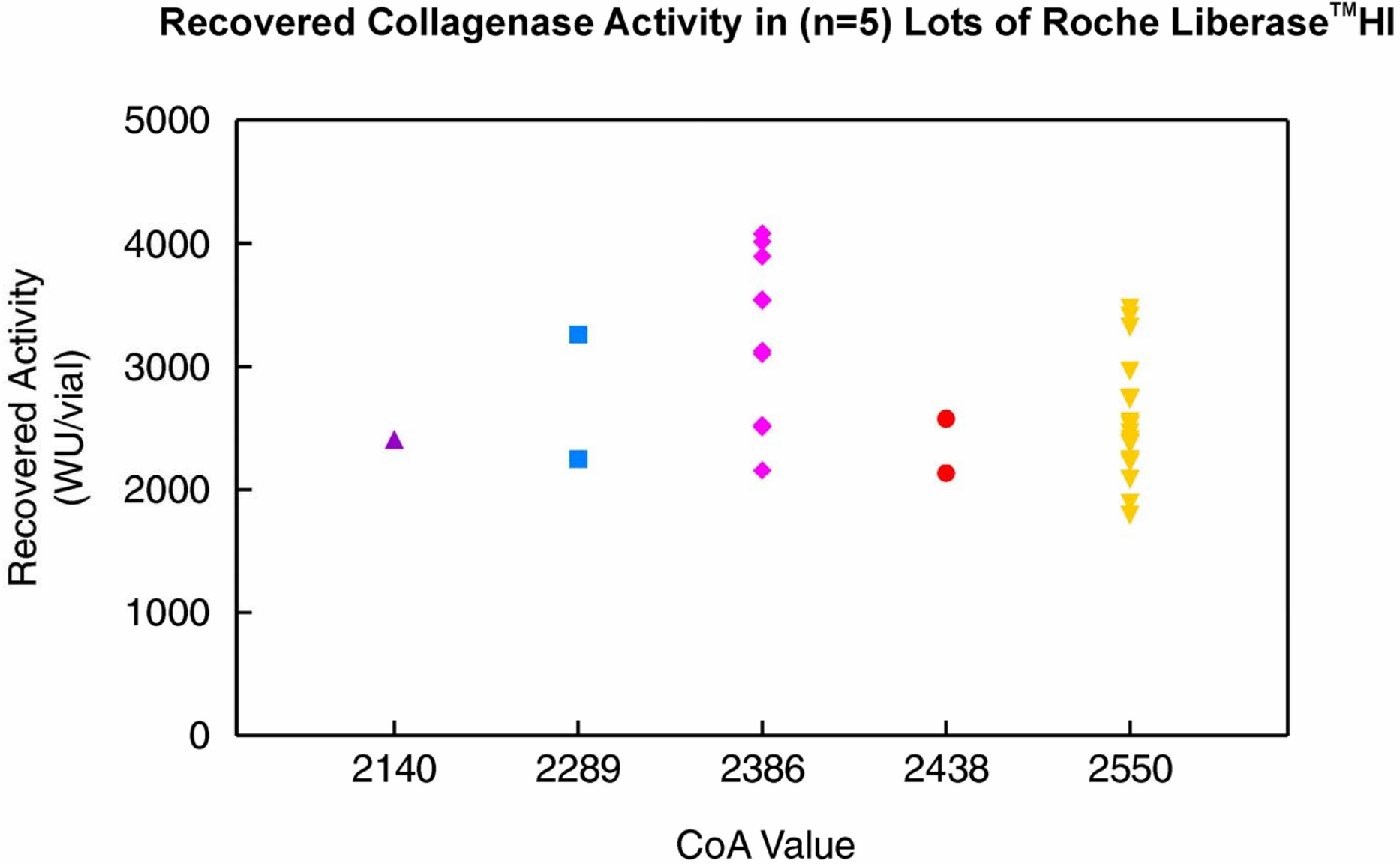
N = 42 (Paired Time intervals)	CI Activity (CDU/µg)	CIb Activity (CDU/µg)
Mean	**2.33†**	**1.35†**
SEM	1.2	0.5
t-test*	p = 0.00011†	

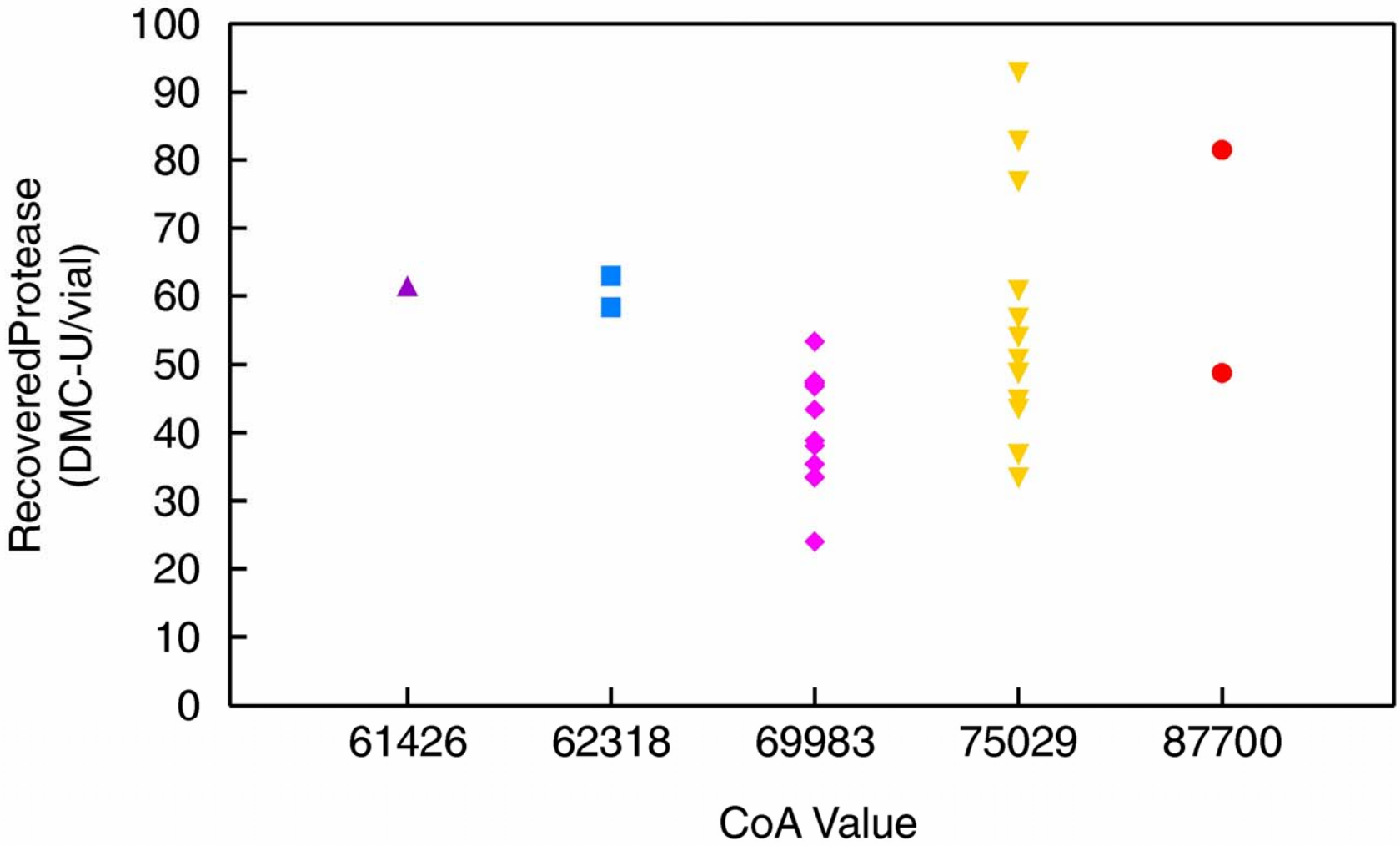
C1b is less potent – poorer performance during digestion

Comparative Isolation Outcomes based on CI Activity

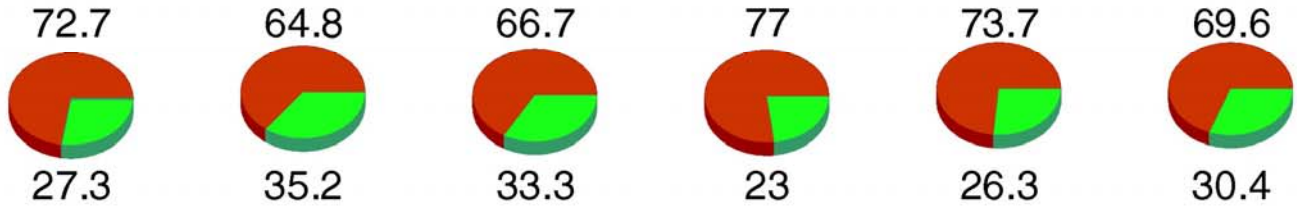
(Where successful >300,000 IE Yield)

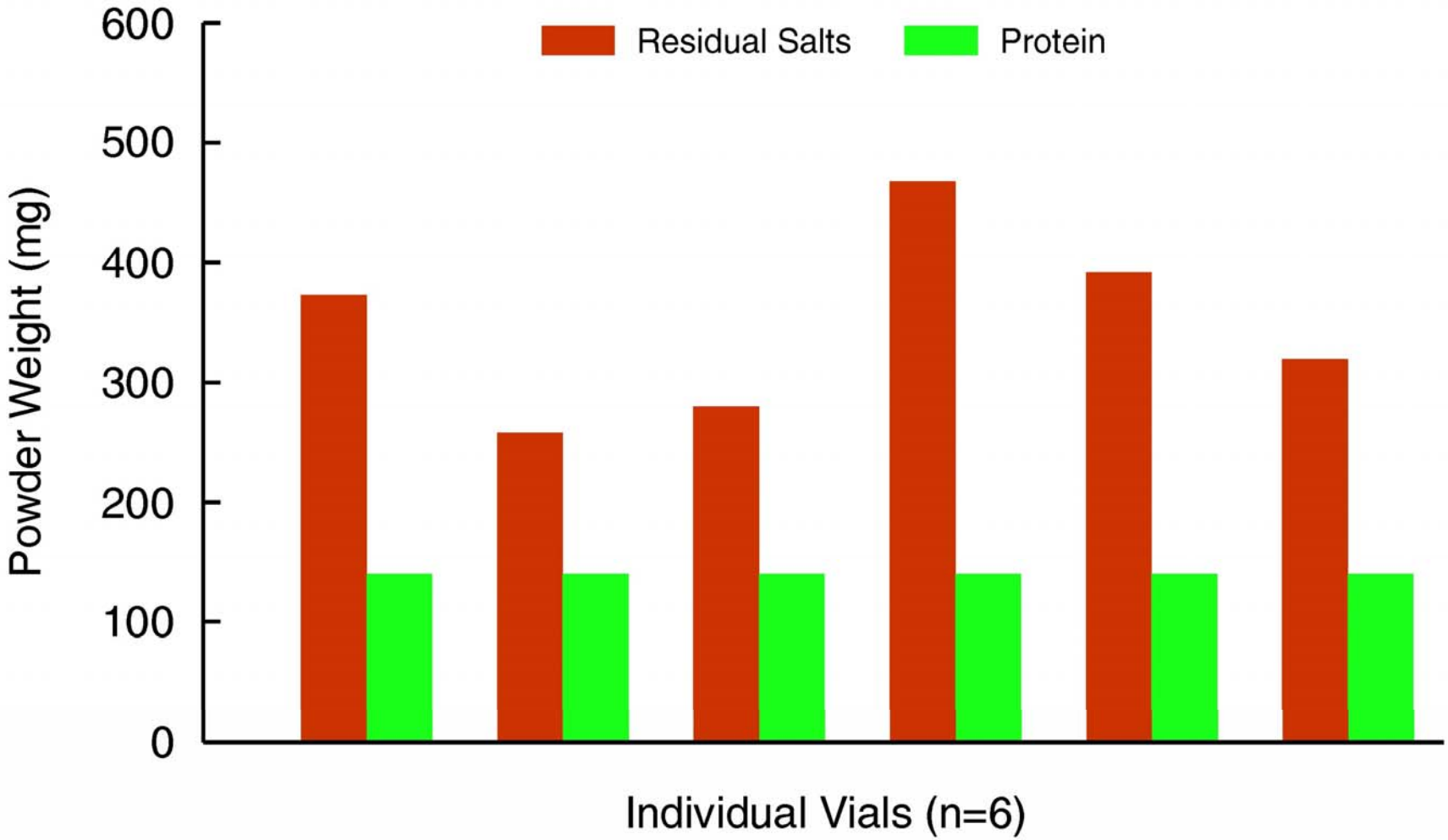
Unsuccessful N=5			Successful N=5	
	CI Activity CDU/ug	**IE Yield**	**CI Activity** CDU/ug	**IE Yield**
Mean	**1.64†**	**177,764 IE**	**2.98†**	**488,075 IE**
SEM	**+/-0.2**	**+/-73,687 IE**	**+/-1.1**	**+/-122,769 IE**
t-test	***p= 0.0447†*** **one-tailed, paired: p<0.05**			


Recovered Collagenase Activity in (n=5) Lots of Roche Liberase™HI
Recovered Activity (WU/vial)
5000
4000
3000
2000
1000
0
2140
2289
2386
2438
2550
CoA Value


Recovered Protease Activity in (n=5) Lots of Roche Liberase™HI
RecoveredProtease (DMC-U/vial)
100
90
80
70
60
50
40
30
20
10
0
61426
62318
69983
75029
87700
CoA Value

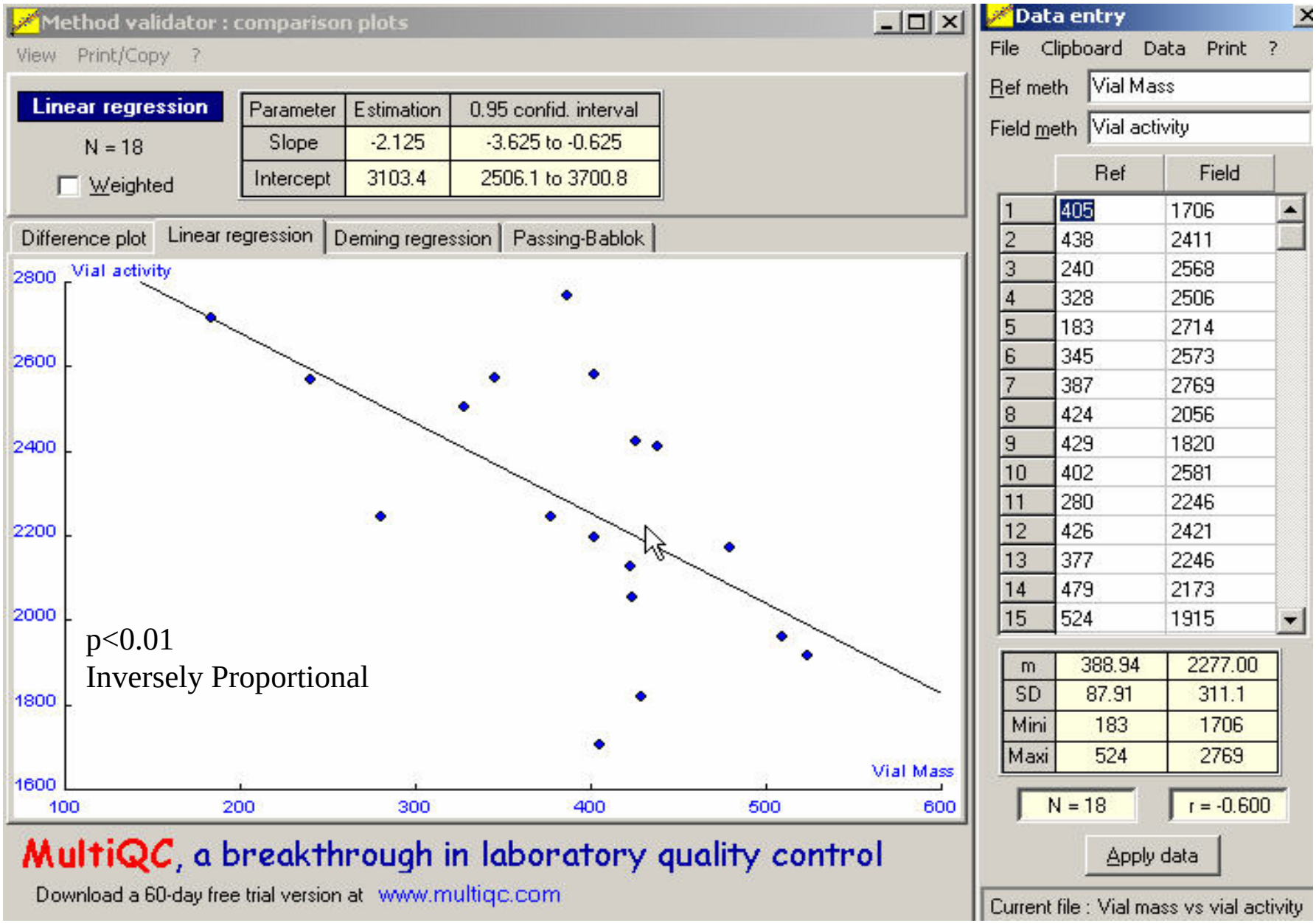
Intra-lot Mass-Protein Distribution in Roche Liberase™HI



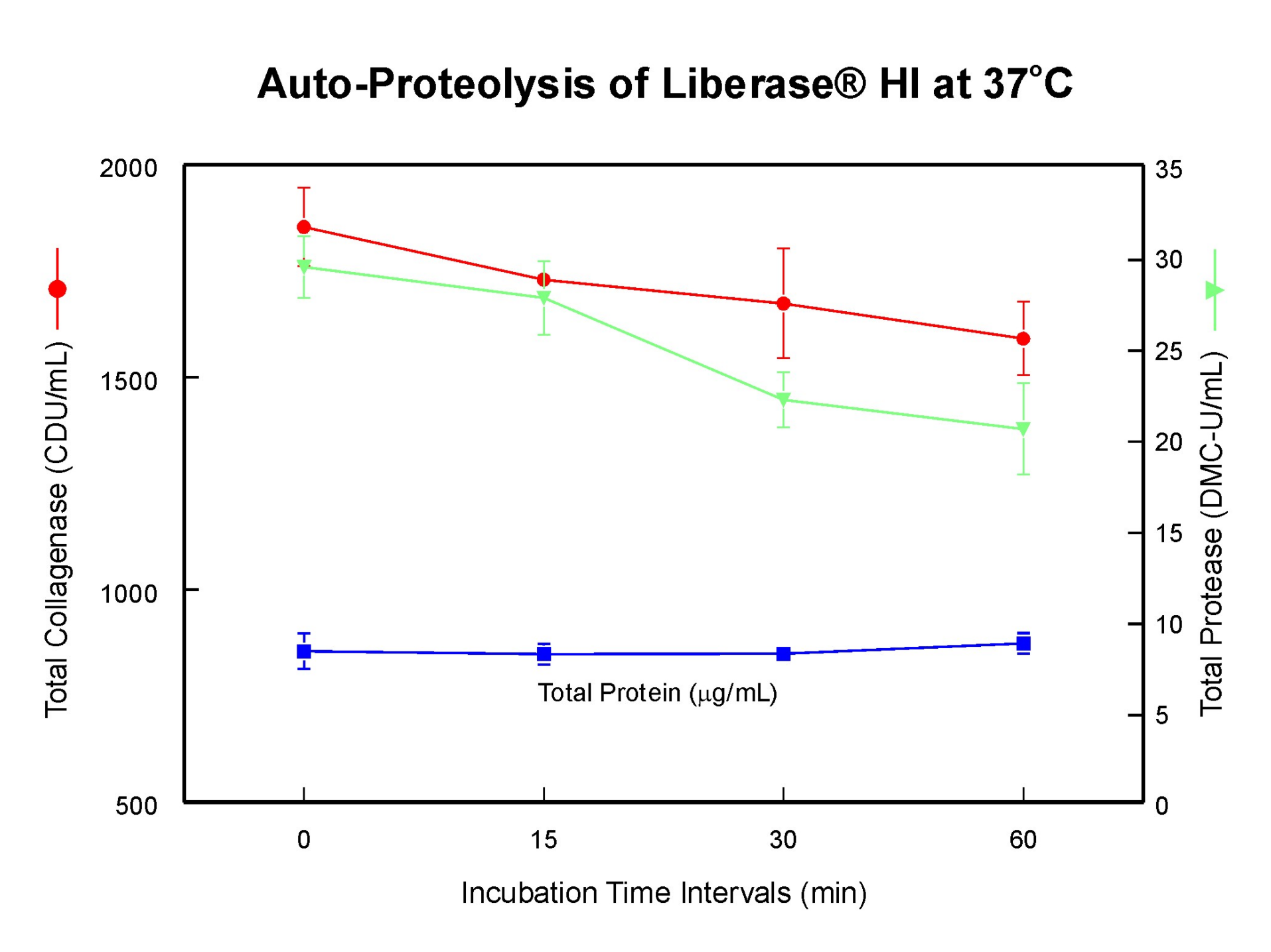


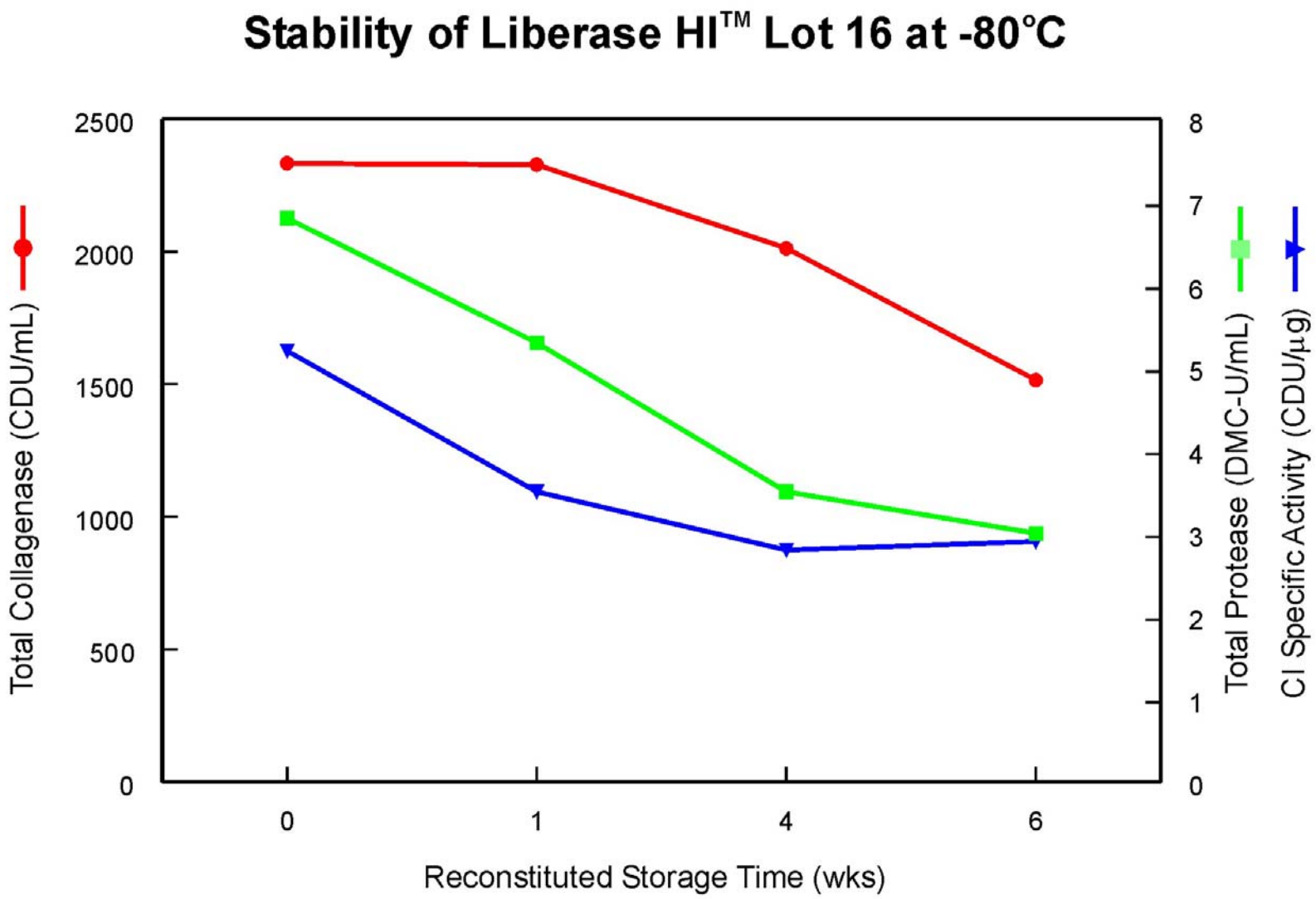
Roche CoA Lot 93266020 **77,538 NP/Vial**	Powder Mass (mg)	**Specific Activity** (DMC-U/mg)	Recovered Activity (NP/Vial)
Mean +/- **SEM** **Range**	48.1 +/- 11.7 **30.8-70.0**	70.9 +/- 16.3 **45.7-93.1**	61,208 +/- 10,931 **50,539 - 75,808**

Lot 93290120 Vial Mass vs. Collagenase Vial Activity

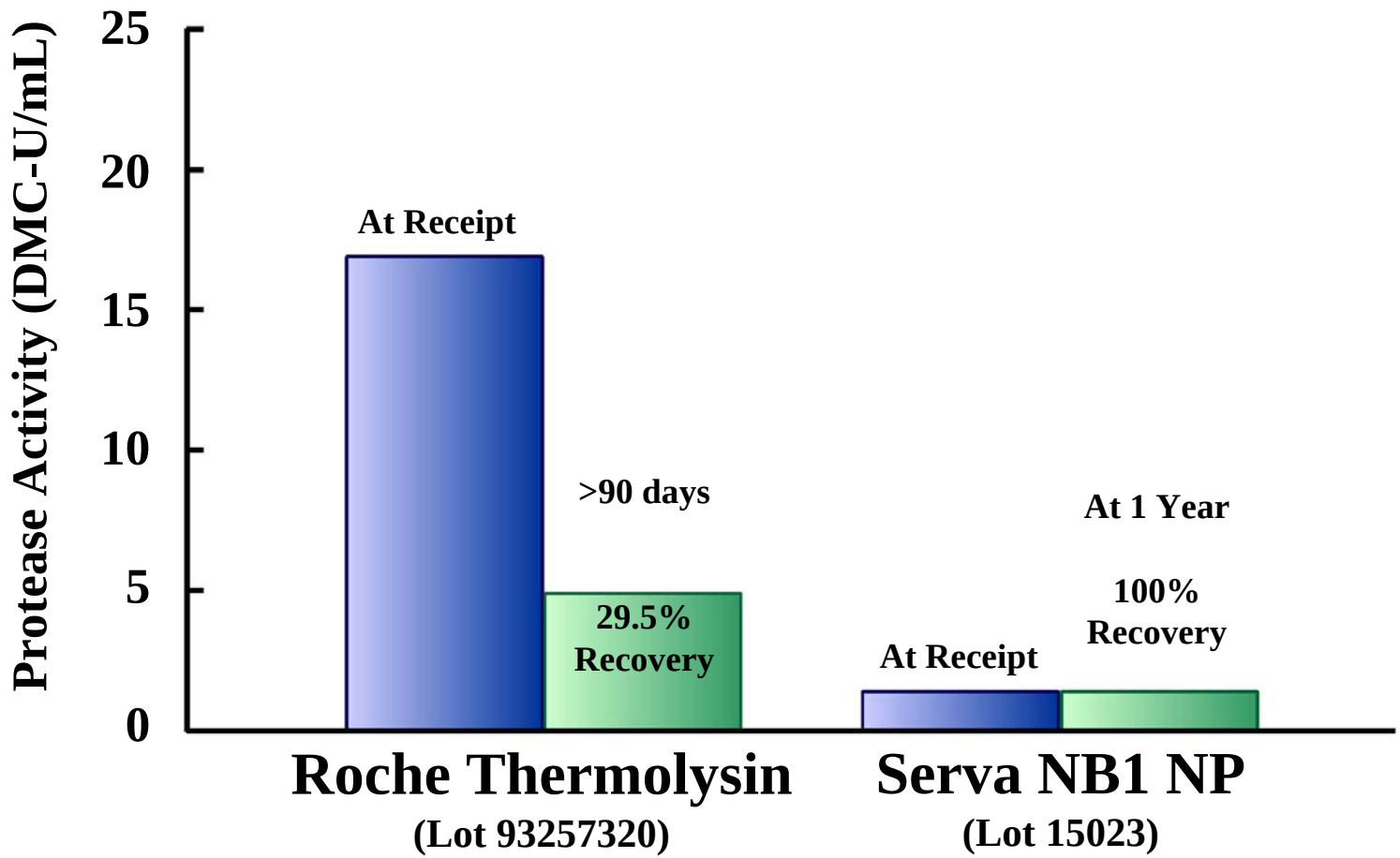


Long Term Storage


Auto-Proteolysis of Liberase® HI at 37°C
Total Collagenase (CDU/mL)
Total Protease (DMC-U/mL)
Total Protein (μg/mL)
Incubation Time Intervals (min)
2000
1500
1000
500
35
30
25
20
15
10
5
0
0
15
30
60


Stability of Liberase HI™ Lot 16 at -80°C
Total Collagenase (CDU/mL)
2500
2000
1500
1000
500
0
8
7
6
5
4
3
2
1
0
Total Protease (DMC-U/mL)
CI Specific Activity (CDU/µg)
0
1
4
6
Reconstituted Storage Time (wks)

Comparison of Protease Powder Stability
N=3



Effect of Extended Digestion Time On Yield Outcome

Outcome*	Mean IE Yield	Mean Dilution Time (min)
Successful (n=15)	510,417 ± 146,817	16.9 ± 1.9
Unsuccessful (n=13)	209,834 ± 58,772	20.0 ± 5.0

Where a successful outcome is defined as a pre-purification yield of > 300,000 IE.

ACKNOWLEDGMENTS

AMJ Shapiro
E. Ryan
P. Senior
B. Paty.
D. Bigam

N. Kneteman
R. Owen
K. O'Kelly

D. McGhee-Wilson
Dr T Kin
R. Wilson
M. Barnett
D. O'Gorman
S. Rosichuk
B. Richer
T. Murdoch

W Zhai
R Pawlick
G Woldridge

C. Ricordi B. Hering

JDRF dedicated to finding a cure

Capital Health

Alberta GOVERNMENT OF ALBERTA

United States National Institute of Diabetes & Digestive & Kidney Diseases of the National Institutes of Health

Immune Tolerance Network

AHFMR ALBERTA HERITAGE FOUNDATION FOR MEDICAL RESEARCH

25 YEARS OF EXCELLENCE

UNIVERSITY OF ALBERTA QUAECUMQUE VERA

Liberase HI Ordering Options

- We would like to work with any research group looking for customized products for tissue dissociation and cellular isolation
- Please contact:

Jeffrey Emch

Product Manager

Roche Applied Science

1-800-428-5433 x 12760

Jeffrey.emch@roche.com

Liberase HI Ordering Options

Standard:

- Liberase HI Cat# 11666720001 0.5g
  - Multiple lots available for selection
  - We will hold material of a particular lot with a PO
  - Split shipping for evaluation available

Custom:

- Collagnease Blend Cat# 03667430001
- Thermolysin Cat# 03667421001
  - Produced upon reciept of PO
  - 4-6 weeks delivery
  - Same specifications as standard product

Continued Improvement of Liberase HI

- Longstanding relationships and communication with the Islet isolation research community
  - Your feedback and recommendations and custom requests are appreciated
- New larger capacity lyophilizer will allow for larger lot size to improve availability of material (available now)
- New processes and equipment for use in filling under evaluation to address lyophilized mass variances
- Custom options available to address specific needs
- cGMP Collagenase available August 2005 – *this is not Liberase HI or the existing RAS Collagenase blend*