VitaCyte®
Unravelling Cell Isolation

**Webinar**

# Collagenase Selection & Process Optimization for High Yield Hepatocyte Isolation

**Rafal P. Witek PhD, MBA**
Senior Director of R&D Engineering
Dimension Inx

# Agenda

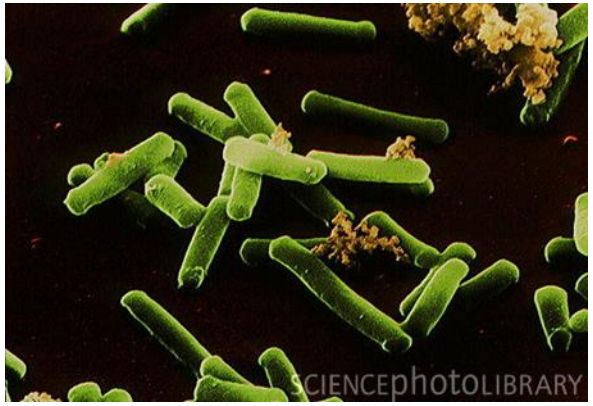
- **Introduction and strategy for choosing Collagenase**
- **Hepatocyte Isolation and process optimization**
- **How to get most cells out of the liver - examples**


*Disclosure: All data in this presentation has been publicly presented and/or do not include any proprietary or confidential information

# Collagenase - Introduction

## *Clostridium histolyticum*

- An anaerobic, motile, gram-positive bacterium that thrives in feces and soil
- The ammonia and proteases it produces, including several collagenases, digest proteins outside its body into amino acids, which it eats.



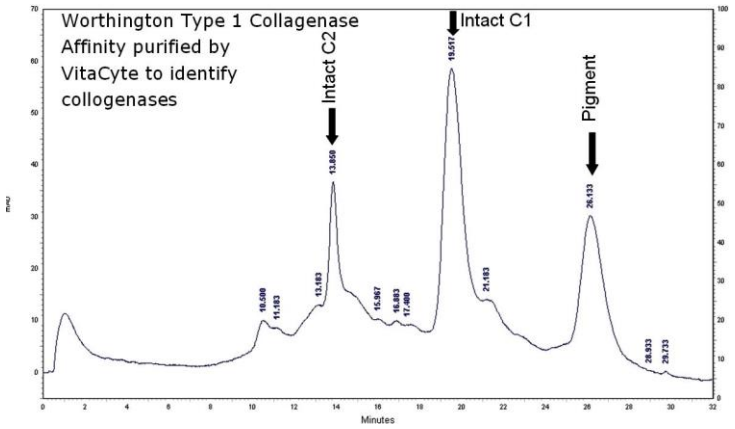

**Crude collagenase** is minimally purified *Clostridium histolyticum* culture supernatant that is later lyophilized: **high lot to lot variability**

Contains essential enzyme for tissue dissociation; collagenase and neutral protease but also other components: other enzymes, endotoxin, pigment

**Enriched collagenase** products are further purified to reduce pigment and increase enzyme activity but still reflects **lot to lot variability** of the culture supernatant

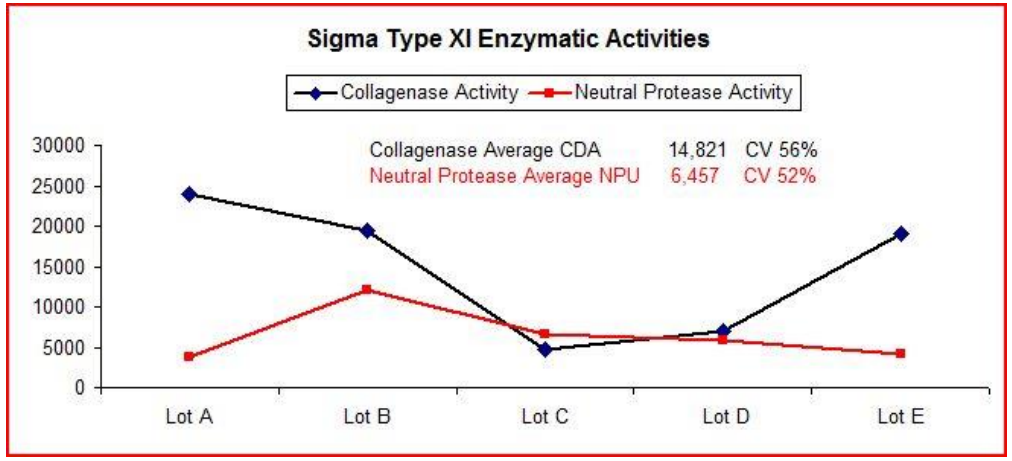
## Key biochemical concepts that define Collagenase

**Two classes of collagenase – class I (C1)** and **class II (C2)** – initially defined by different substrate specificities, degrade collagen synergistically




**Crude collagenase**
(HPLC)

Collagen degradation activity (CDA) is the critical enzymatic activity required for tissue dissociation



## Types of Collagenase

- **Type 1** crude collagenase has the **original balance** of collagenase, caseinase, clostripain and tryptic activities.
- **Type 2** contains **higher** relative levels of protease activity particularly clostripain.
- **Type 3** contains **lowest levels of secondary proteases (that means clostripain and caseinase)**.
- **Type 4** is designed to be especially **low in tryptic** activity to limit damage to membrane proteins and receptors.

- Hepatocytes in a normal non-fibrotic liver are sensitive to Trypsin and can be easily damaged if Collagenase has strong tryptic activity
- Fibrotic livers will require stronger Tryptic activity to release hepatocytes from underlying ECM

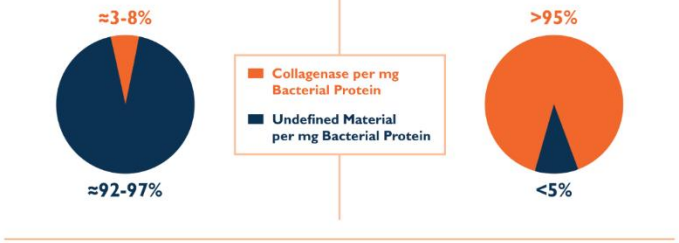
# Collagenase – Strategy for Selection

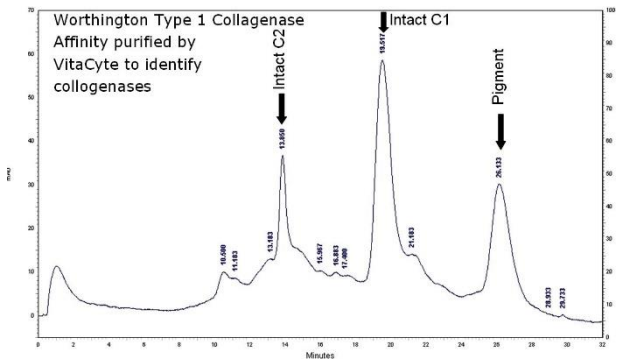
VitaCyte®

Unravelling Cell Isolation

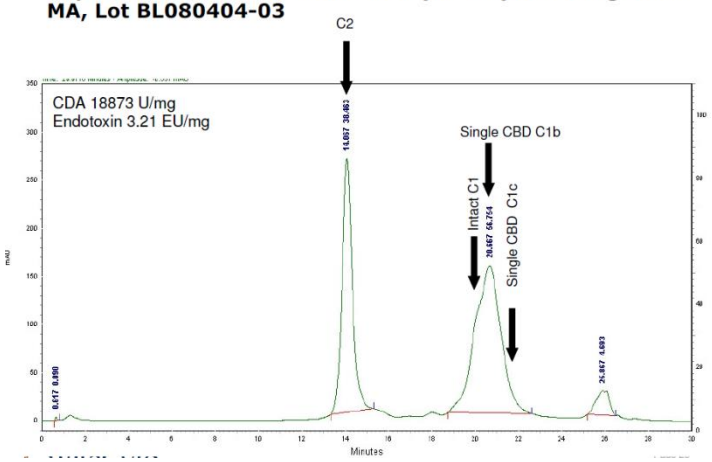
**Traditional Collagenase** vs **VitaCyte Collagenase**

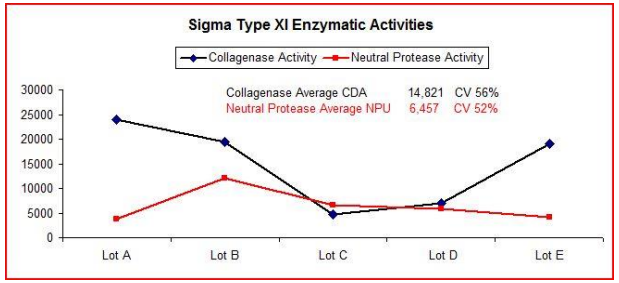
**Purity**

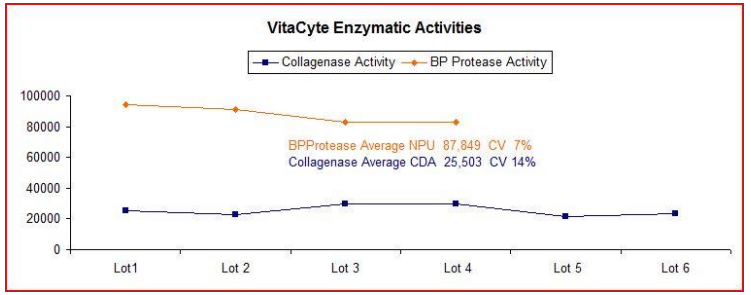



**Representative results from VitaCyte CIzyme Collagenase MA, Lot BL080404-03**



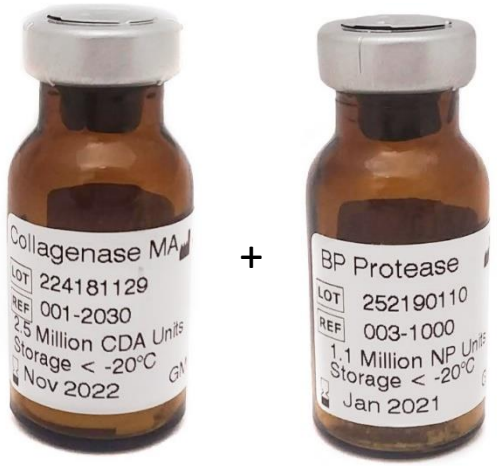




- VitaCyte Collagenase is consistent from batch to batch and has high Collagen degradation activity (CDA)
- **VitaCyte offers ability to optimize isolation process and maximize hepatocyte yield per gm/tissue**

VitaCyte®
Unravelling Cell Isolation

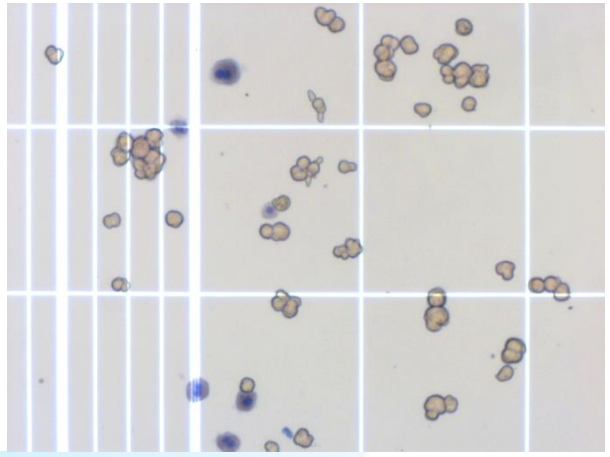
## VitaCyte Working Collagenase = Collagenase MA + BP Protease

**Collagenase** – separation of cells from ECM
**Protease, EGTA** – separation of cells from each other

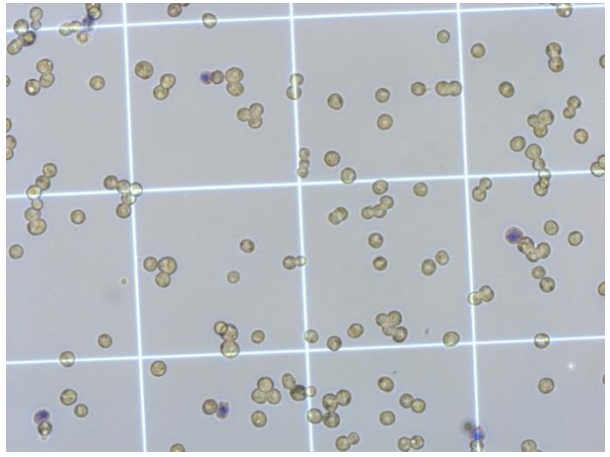



By adjusting perfusion time for Buffer 1 (EGTA) and adjusting concentrations of collagenase and protease, a “perfect” combination of digestive enzymes can be established for all specie hepatocyte isolations that would allow maximum cell recovery (yield) and minimal damage to cellular membranes (viability and cryo).

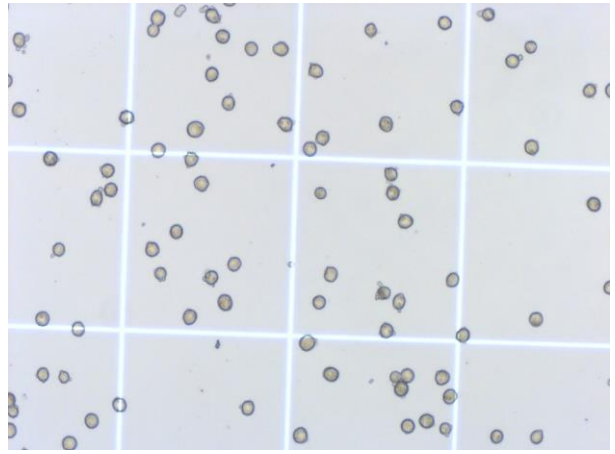
**Mouse**



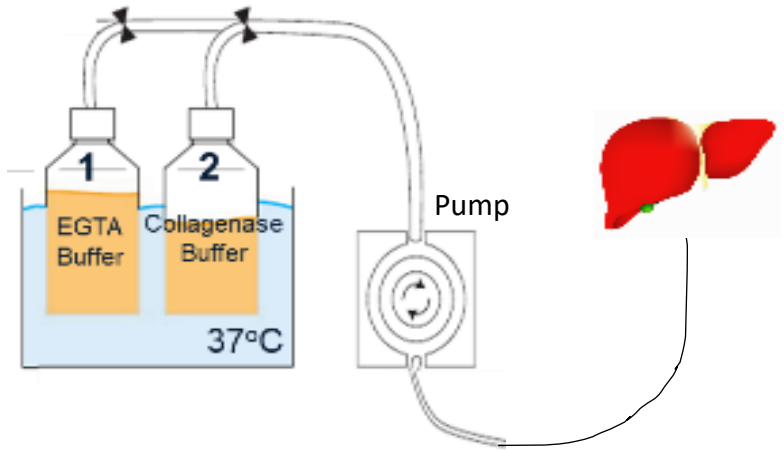
**Rat**



**Human**



# Hepatocyte Isolation - Introduction




## Standard 2 Step Collagenase Isolation of Hepatocytes

**(Step 1) – Perfusion with 37°C Calcium free buffer + EGTA**

- blood removal
- re-inflation of capillaries – perfusion flow speed is based on liver inflation
- relaxation of E-Cadherin junctions by EGTA
- time dependent process

**(Step 2) – Perfusion with 37°C buffer containing Calcium and Collagenase**

- start with Vitacyte recommended enzyme concentrations, then optimize
- time and Collagenase concentration dependent process
- digestions stopped when tissue disaggregation visible
- too short digestion (under-digested) = low yield, low viability (bellow 70%)
- too long digestion (over-digested) = high yield, low viability (bellow 70%)

## Approximate Number of Hepatocytes:

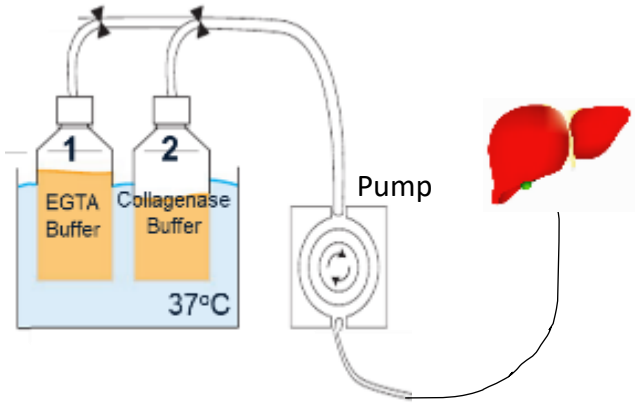
- **Human Liver**: 100-160 Billion Hepatocytes
- **Rat Liver**: 1.5-2 Billion Hepatocytes
- **Mouse Liver**: 100 – 150 Million Hepatocytes

## What to expect in number of isolated hepatocytes if process is optimized:

- **Human Liver**: **flow at 1/3 of tissue mass, 20-25 min**, **20-40 Million /gm** tissue (Adult liver, sectioned), → 20-40 Billion total, 60-80% viability
  **flow at 1/3 of tissue mass, 20-25 min**, **50-75 Million/gm** tissue (pediatric, sectioned or whole) → 15-25 Billion total, 80-95%
- **Rat Liver**: **15-20ml/min, 15-20 min**, **0.8-1 Billion** Hepatocytes per rat, 80-95% viability
- **Mouse Liver**: **6-10ml/min, 10-12 min**, **50-100 Million** Hepatocytes per mouse, 80-90% viability

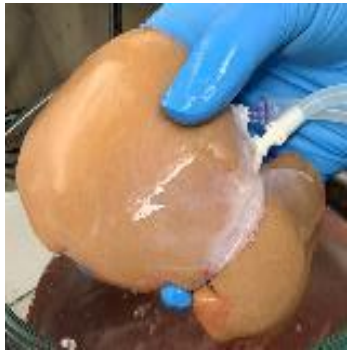
# Hepatocyte Isolation – High Level Summary

## *Ex vivo* Isolation Method




- Enzymes selection
- Type of isolation buffers
- Whole organ vs resection perfusion
- Single pass vs recirculation

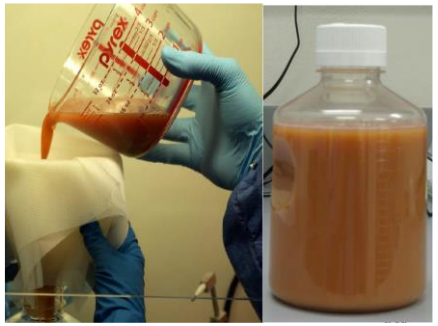
## Liver Tissue



- Tissue acceptance criteria
- Donor age
- Tissue "quality"
- WIT/CIT

Digestion

## Hepatocyte preparation



- Process variations
- Purification methods
- Immediate processing vs recovery
- Temperature of processing

## Cryopreservation

- Crygenic solution
- Handling and Storage (Vapor Phase LN2)
- Vial to vail variability
- Shipping conditions

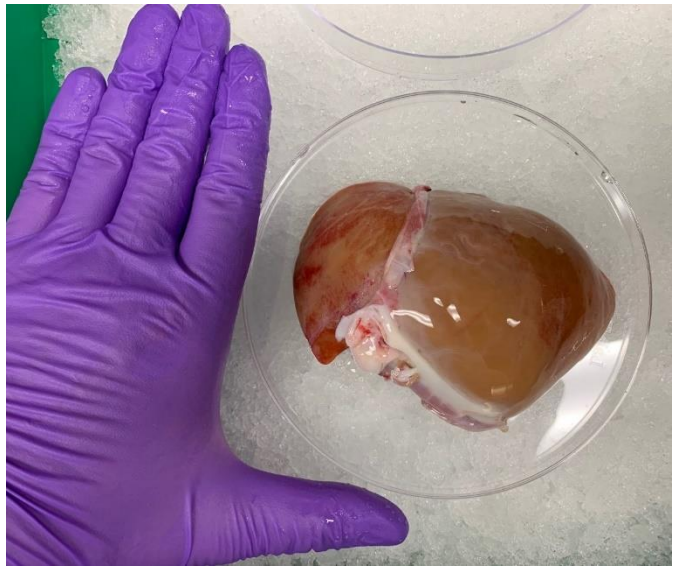
- Isolation of primary human hepatocytes is a **highly variable process** that can substantially differ from group to group
- **Each isolation process is "unique"**
- Industry developed analytical methods to help standardize selection of good quality lots for their customers – CofA

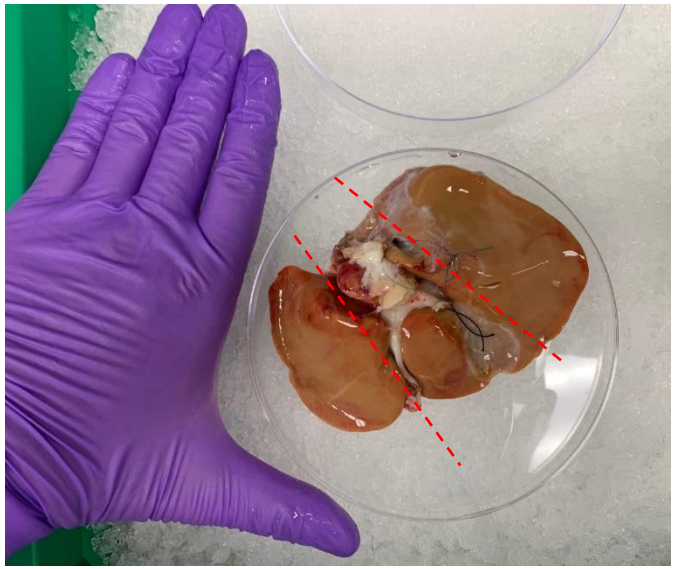
# Liver preparation – Pediatric, cut, Vitacyte Collagenase MA

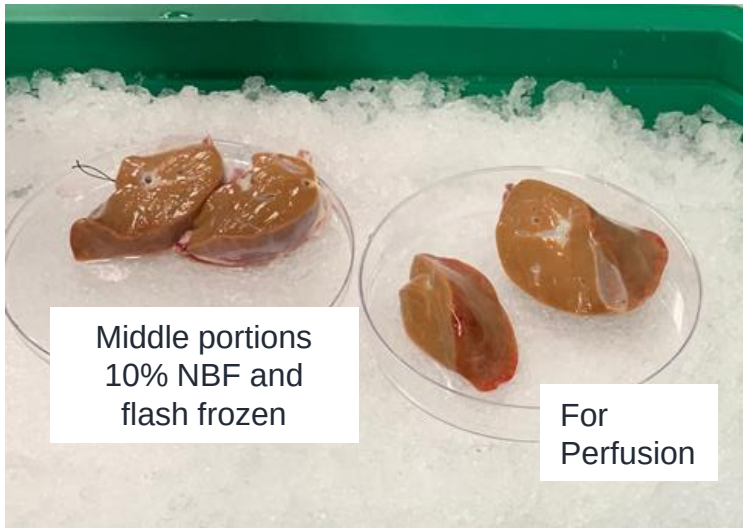
**Pediatric Liver**
BMI: 22.3
Tissue Weight: 150gm,
100gm perfused – 35ml/min

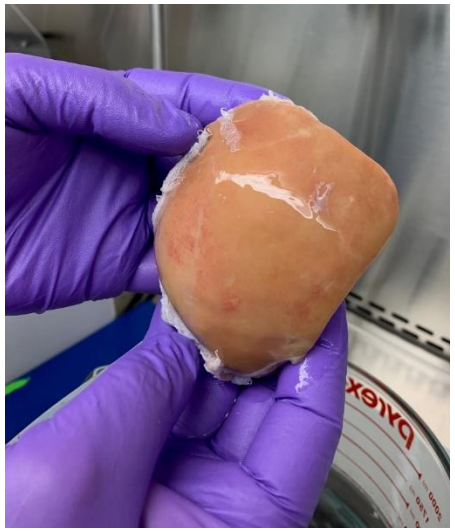
**Post Isolation**
Viability post isolation: 85%
Yield post isolation: 8.9 Billion (89 million/gm tissue)

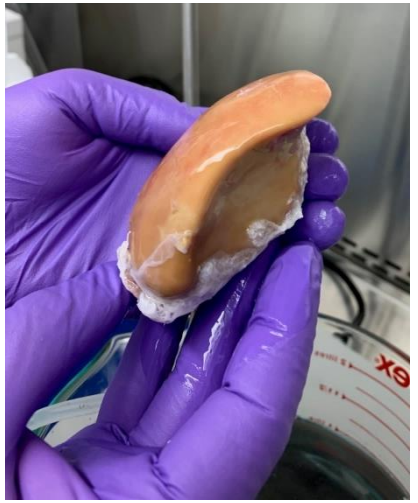
Final Viability after cleanup: 93.2%
Final yield: 7.27 Billion (72 million/gm tissue)







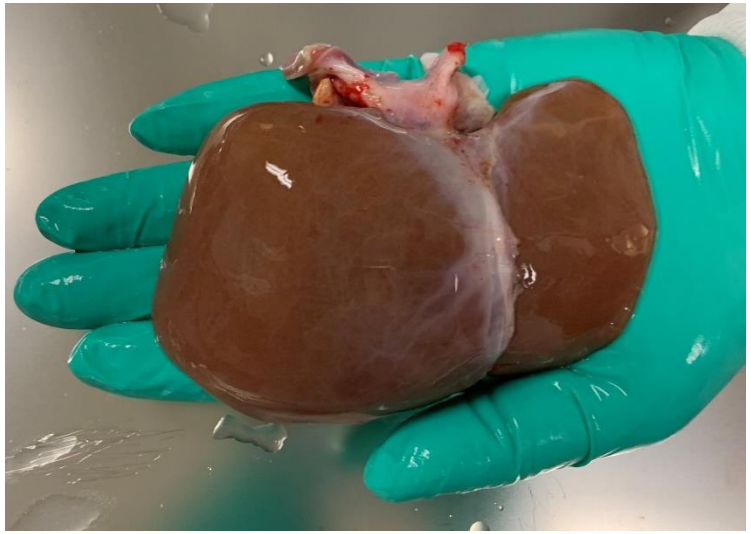


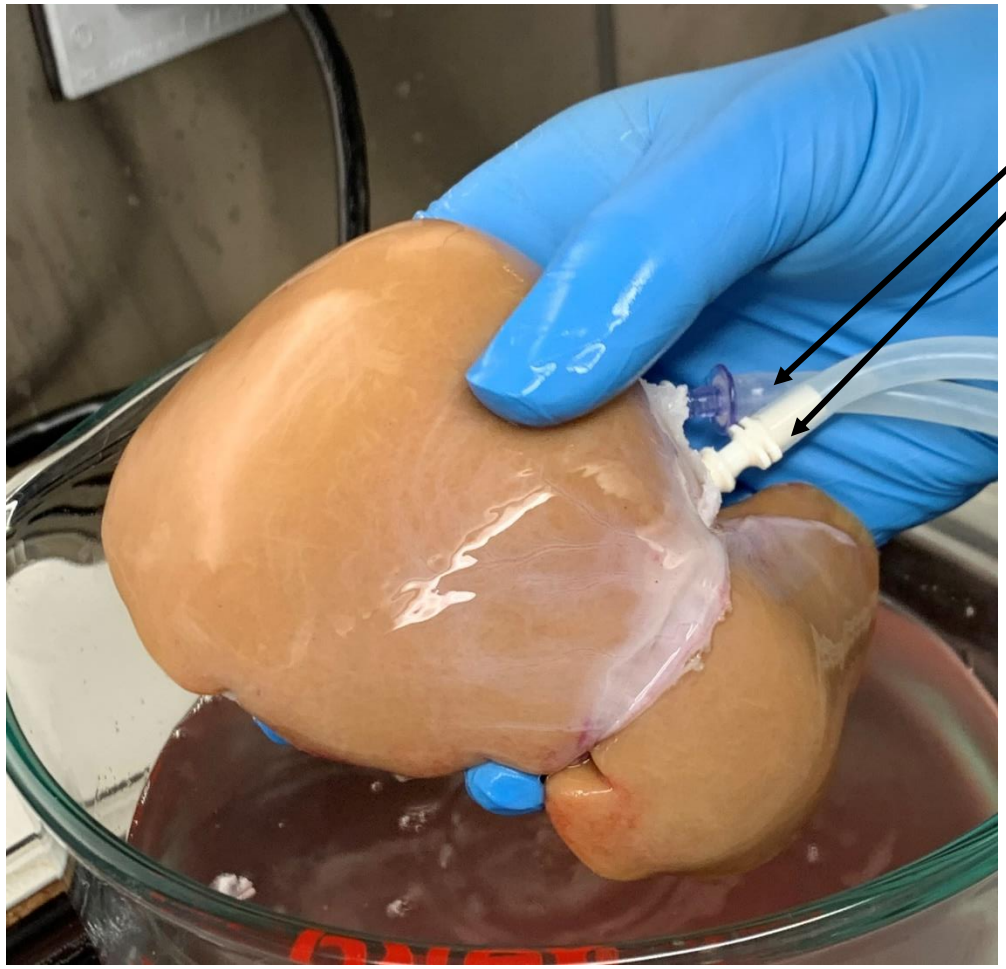
# Liver preparation – Pediatric, whole, Vitacyte Collagenase MA

**Pediatric Liver**
BMI: 21
Tissue Weight: 240gm – 80ml/min

**Post Isolation**
Viability post isolation: 89%
Yield post isolation: 16.8 Billion (70 million/gm tissue)

Final Viability after cleanup: 96%
Final yield: 15.2 Billion (63 million/gm tissue)





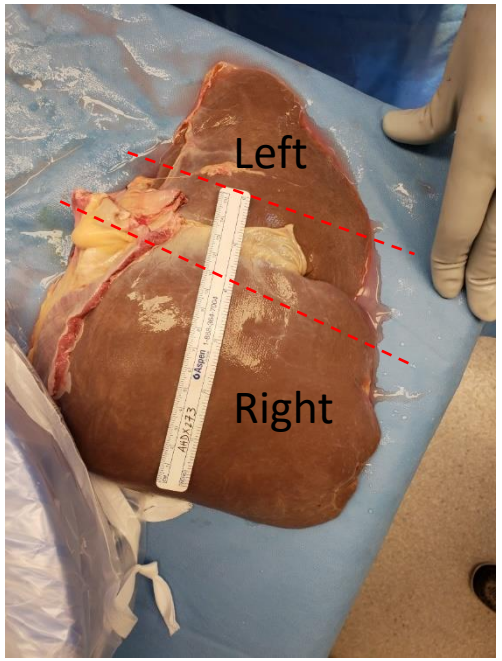
# Liver preparation – Adult, cut, Vitacyte Collagenase MA

**Adult Liver**
BMI: 25.7
Tissue Weight: 1,450gm

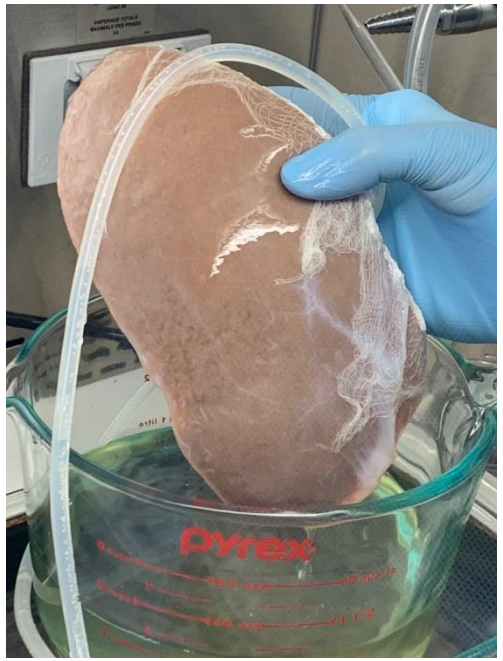
Left Lobe: 280gm – 100 ml/min
Right Lobe: 670gm – 140ml/min
Total perfused: 950gm

**Post Isolation**
Viability post isolation: 76%
Yield post isolation: 36 Billion (38 million/gm tissue)

Final Viability after cleanup: 92%
Final yield: 24.2 Billion (25 million/gm tissue)



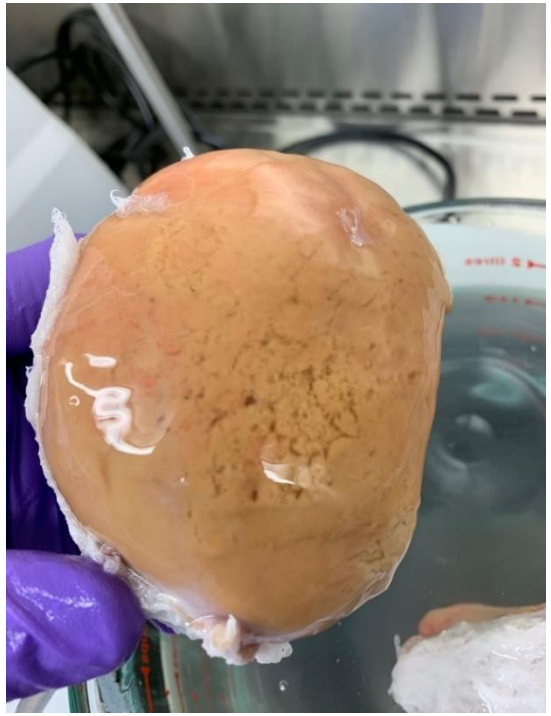

Cut and sealed, ready for perfusion



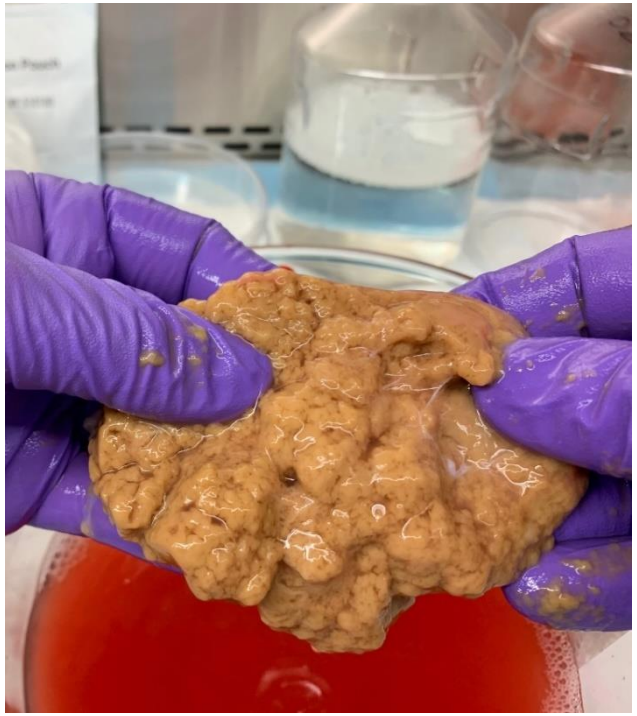

Right lobe during perfusion

10B hepatocytes in 2L bottle

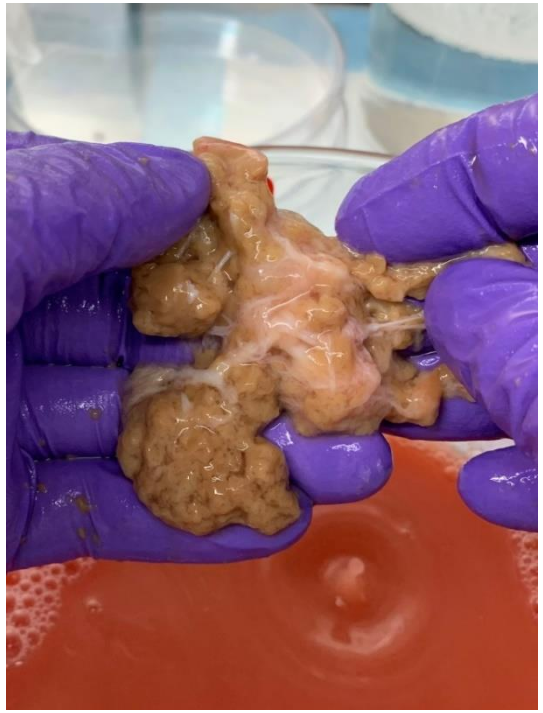
# Liver digestion – how to tell when digestion is completed



Tissue breakage indicates complete digestion



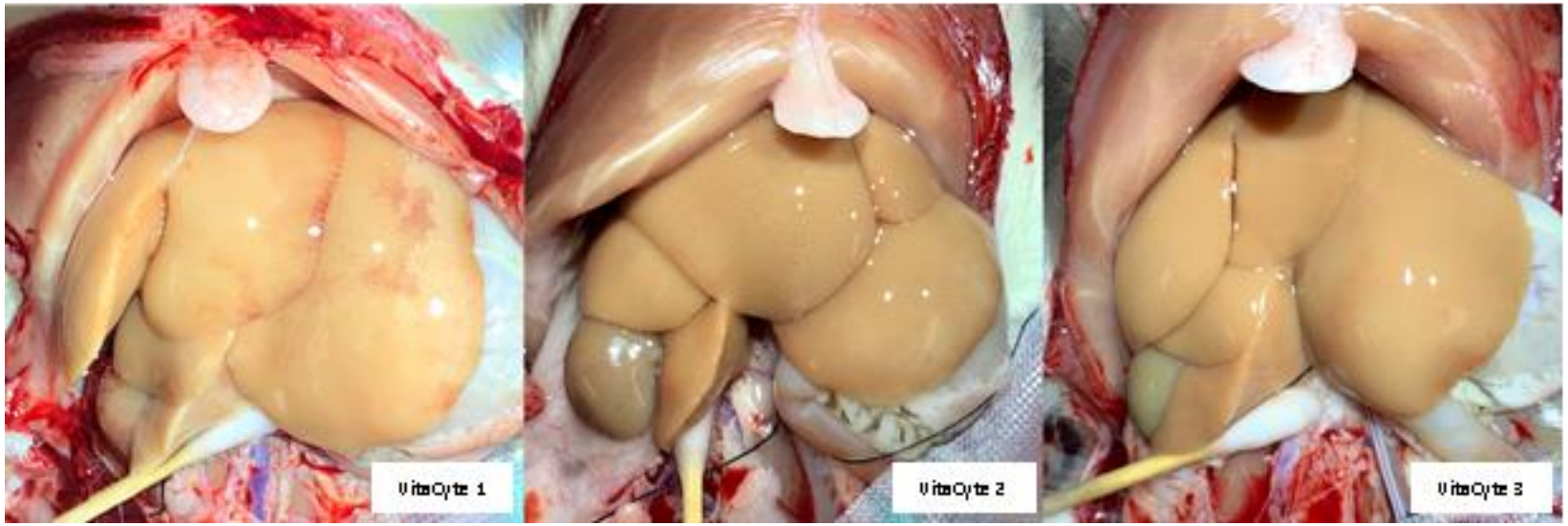
- Tissue disaggregation is uniform, cells fall apart easily,
- Need 3-5 min to shake the cells off the scaffold



- Visible ECM and nearly full tissue disaggregation

- 1,200 vials of hepatocytes at 15M hepatocytes/vial

# Liver digestion – Rat isolation, Vitacyte MA Collagenase




| | | | Pre-Percoll® | | | | Post-Percoll® | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver | Liver mass (g) | Enzyme | Total yield | Viable yield | Viability | Viable yield/ g liver | Total yield | Viable yield | Viability | Viable yield/ g liver |
| 1 | 15.4 | VitaCyte® (MA/BP) | 7.80E+08 | 6.30E+08 | 0.81 | 4.09E+07 | 3.23E+08 | 2.95E+08 | 0.91 | 1.92E+07 |
| 2 | 13.5 | | 1.11E+09 | 9.90E+08 | 0.89 | 7.33E+07 | 9.00E+08 | 8.35E+08 | 0.93 | 6.19E+07 |
| 3 | 12.8 | | 8.63E+08 | 6.90E+08 | 0.80 | 5.39E+07 | 6.30E+08 | 6.00E+08 | 0.95 | 4.69E+07 |

## Conclusion

VitaCyte®
Unravelling Cell Isolation


- Hepatocyte isolation is a highly variable process
- The key to successful high yield isolation is to control variables
- Collagenase is a variable that can be controlled by using Vitacyte Collagenase
- Use of Vitacyte Collagenase allows for controlled process optimization that can result in high yield, high viability isolation process

## Acknowledgments:

ThermoFisher SCIENTIFIC

Ambys MEDICINES

Aspect biosystems

life technologies

AnaBios

Dimension Inx