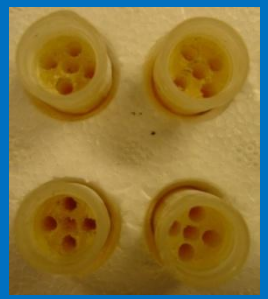




New Method for Processing Banked Samples

Dale Larson

2010 BRN Symposium

March 25, 2010

CRYOXTRACT AN ALLIED MINDS COMPANY

DRAPER LABORATORY

Harvard Medical School

Brown University

Sample Integrity drives the value of a biospecimen and automation facilitates preservation of integrity

Key Automation Drivers[1]

- **Sample Integrity**
- Sample Tracking
- Speed of Access
- Sheer Quantity
- Cost of Manual Access
- Health & Safety

- ***Safeguard*** biospecimen
- ***Reduce*** chance of ***damage*** to biospecimen

=

- ***Increase Value*** of a collection
- ***Increase Trust*** in research results

[1] HTStec. Automated Biobanking Trends. 2006

We developed a Frozen Sample Aliquotter to protect valuable biospecimens

Sample Integrity is hard to control

- Freezing samples to avoid degradation is ubiquitous
- Current sample-handling incurs trade-off between cost and sample integrity

Single-Vial

- Freeze one vial, extract cores as required (thaw), and refreeze remainder
- **Benefits**: lower storage & up front processing costs
- **Drawbacks**: Degradation to sample

Multiple Vials

- Pre-aliquot into small size vials before freezing
- **Benefits**: safeguard sample integrity
- **Drawbacks**: Increased storage requirements and up front costs

Frozen Sample Aliquotter eliminates trade-off & increases sample-handling efficiency

Frozen Sample Aliquotter enables automated extraction of multiple frozen aliquots from one vial of frozen biospecimen

Concept

- Eliminates freeze-thaw cycling
- Automates processes
- Eliminates need for pre-aliquotting

Uses

- Serum & plasma
- Cells
- Small molecule compounds in DMSO
- Frozen Tissues
  - A “Macro Dissection”

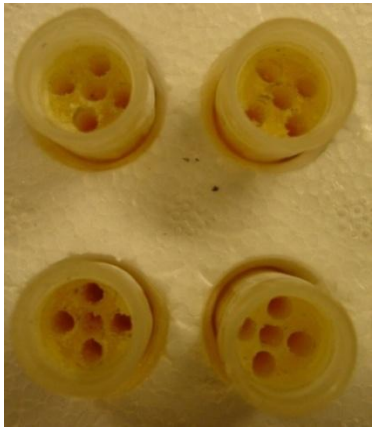
Built and tested basic serum & plasma functionality in “alpha” prototype

CRYOXTRACT DRAPER LABORATORY

Technology is in essence a specialized rotary drilling system

- Stainless steel drill bit with cutting profile drills a core from a specimen under cryogenic conditions and ejects it from the needle into a separate cryovial for downstream analysis
  - Core remains frozen throughout the process

- Key design conditions support modern biobanking
  - Maintain samples below -40°C pre-, during-, post-coring
  - Extract multiple aliquots from 1 vial
  - Achieve volumetric consistency (100µL)
  - Ensure no carryover between samples
  - Deliver hands-free operation

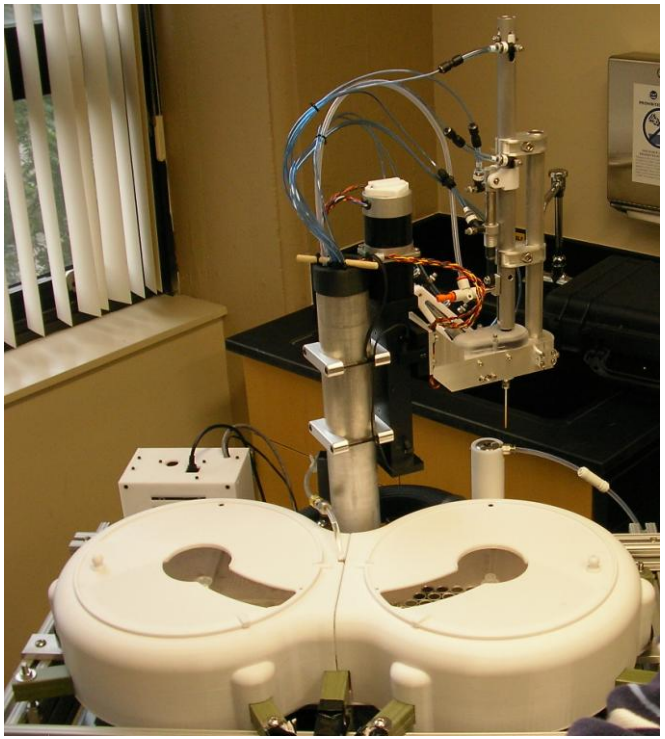


System prototype completed successful 3rd-party testing

CRYOXTRACT
DRAPER LABORATORY

Initial performance testing was successful (R.I. BioBank, Brown U.)

- Frozen Sample Aliquotter can extract multiple, frozen, uniformly-sized and consistently homogeneous portions of plasma, which, when analyzed for common analytes, give reproducible results with very low variability

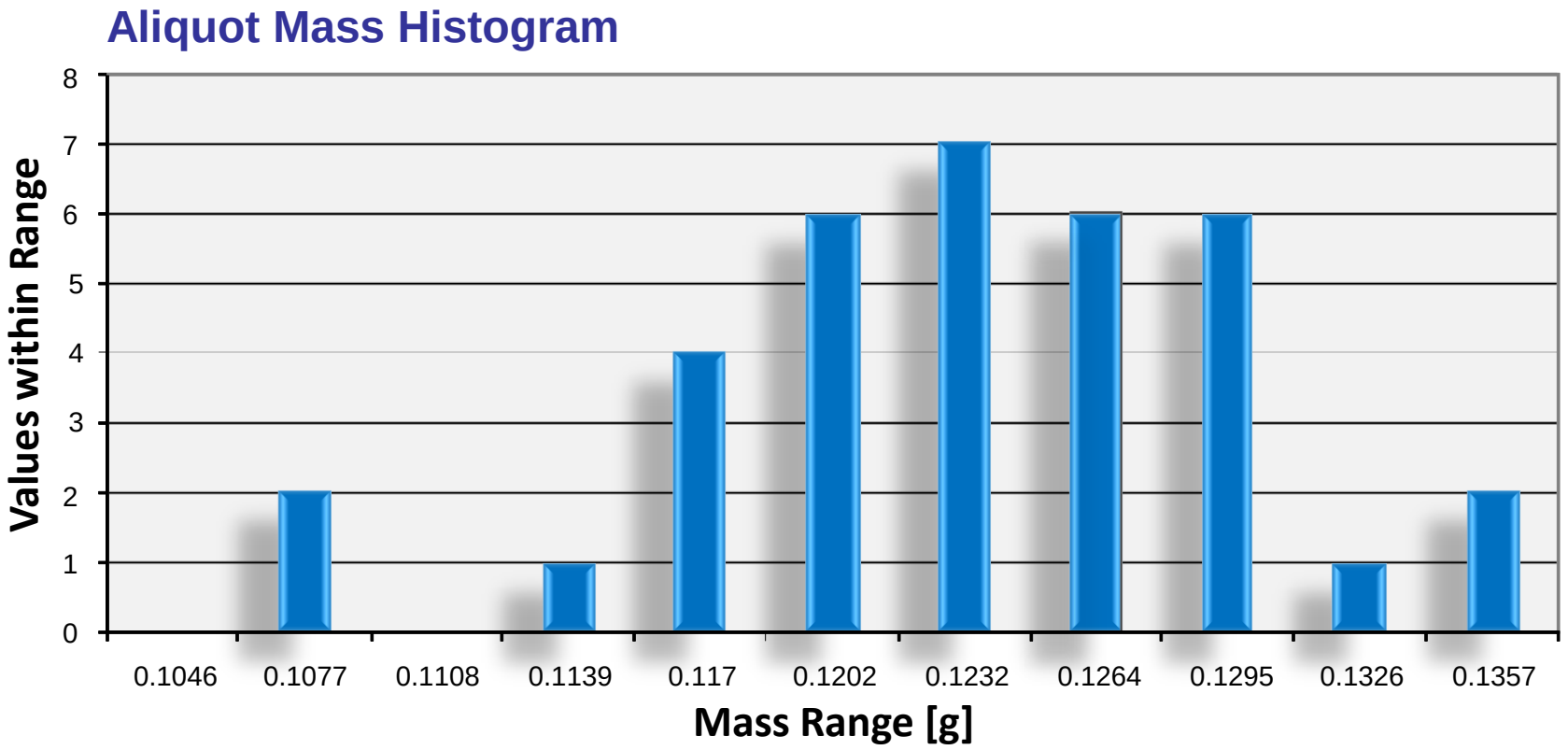


Robot tested at Rhode Island BioBank at Brown University

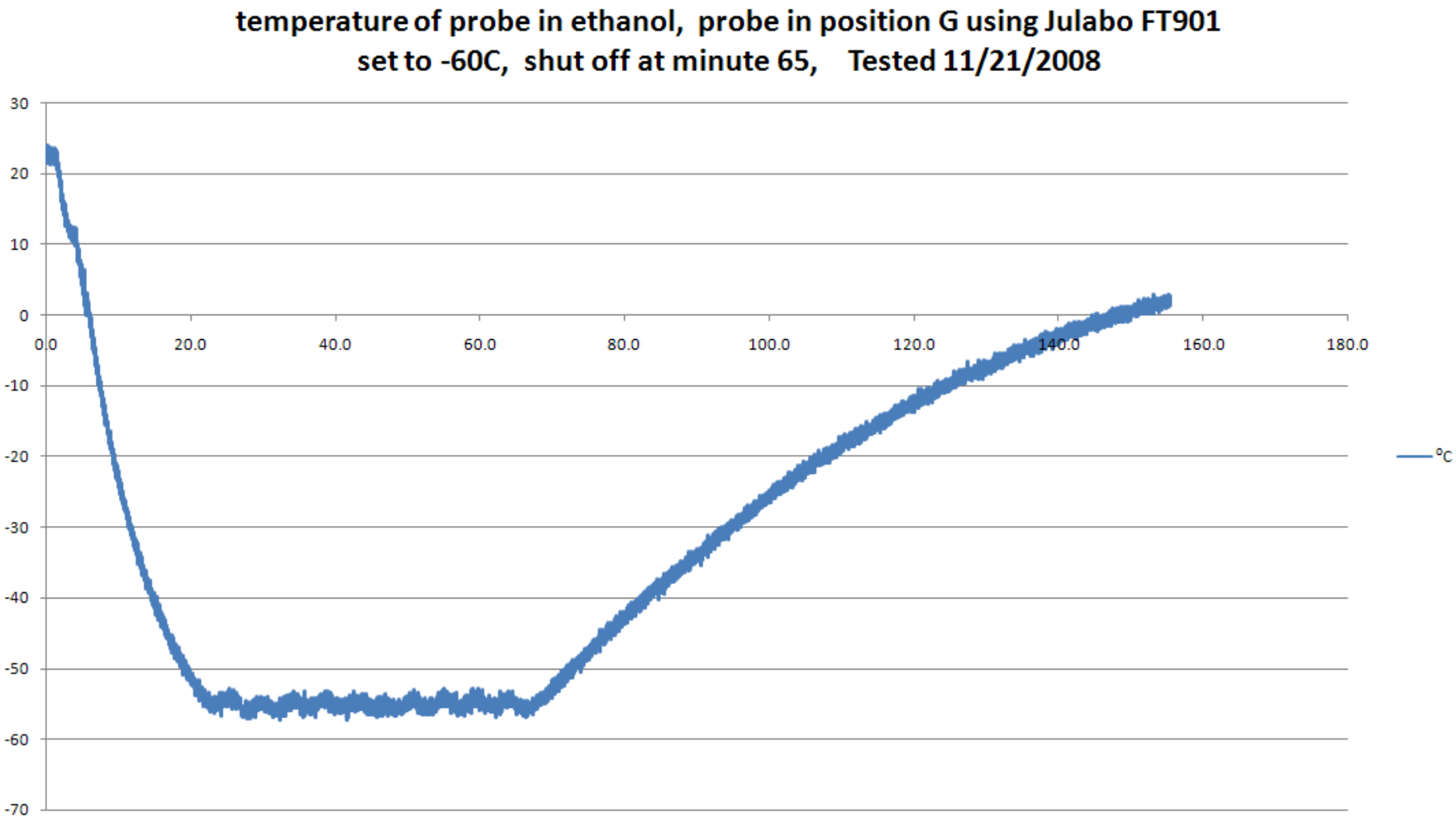
Launched work on second prototype

CV of Aliquot Volume is 5.5%, n=35

- Design point 120μl accounts for under filled tubes
- Measured gravimetrically



Test Data for Fixture Using Immersion Cooler



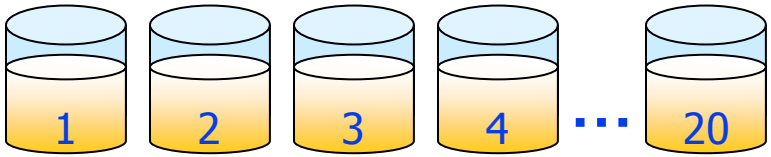
Addressed Carryover Successfully

- Conducted real-time PCR testing to quantify the carryover between samples
- Ran experiment at different levels to simulate multiple sample types
- 10 PCR cycles is equivalent to a reduction of 2^{10} (4096) less genetic material

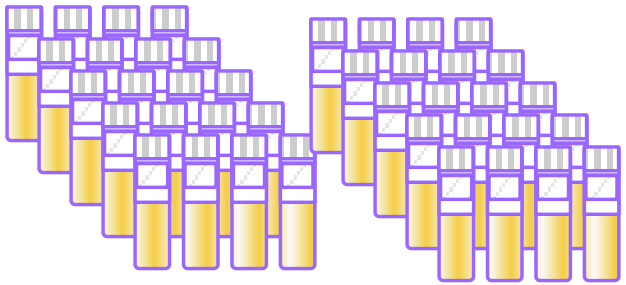
Test	Concentration Level [Cell Equivalents]	Attenuation [PCR Cycles]
1	45,000	10
2		10
3	319,000	14
4		13
5		13
6		13
7	2,300,000	15
8		15
9		15
10	16,000,000	18
11		18
12		17

Sample Preparation for Coring

- Purchased Frozen Plasma
  - 20 Donors, Men & Women, 25 to 55 Yrs Old



- Prepared 40 Source Vials- 1.8ml
  - For later coring



- Prepared 10 Source Vials -200 µl
  - To serve as Control Samples

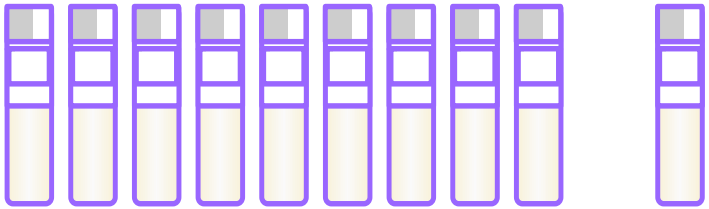
Frozen at -155°C

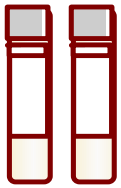
CRYOXTRACT
DRAPER LABORATORY

Reproducibility Study- Sample Preparation

3 Different Donors

10 Source vials from each donor



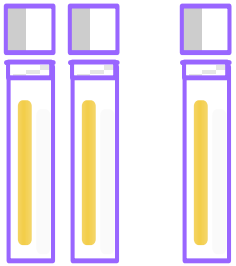


2 Control Vials
from each donor

Extracted 6 cores

"Remainder"

Combined 2 cores
in each destination vial



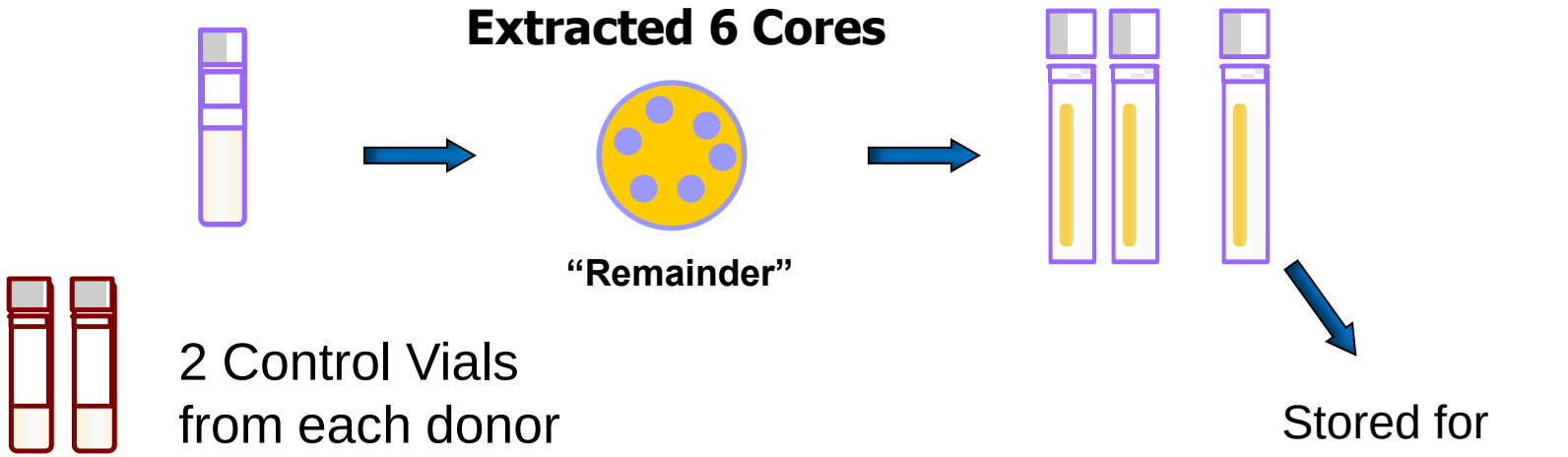
Stored for
future studies

- **72 Vials Sent for Assays**
  - **3 Donors X 10 Source Vials X 2 Destination Vials**
  - **3 Donors X 2 Remainder Vials**
  - **3 Donors X 2 Control Vials**

Diversity Study Sample Preparation

17 Different Donors

1 Source vial from each donor



- **68 Vials Sent for Assays**
  - **17 Donors X 1 Source Vial X 2 Destination Vials**
  - **17 Donors X 1 Remainder Vial**
  - **17 Donors X 2 Control Vials**

Assay Results- Children's Hospital Boston

	T-Chol	Trig	Glucose	IgG	Avg.
Reproducibility Study (few donors, many repeats)					
Cores vs. Controls	**105%**	**108%**	**104%**	**108%**	**106%**
Remainders vs. Controls	**97%**	**100%**	**94%**	**100%**	**98%**
Diversity Study (many donors, few repeats)					
Cores vs. Controls	**101%**	**105%**	**102%**	**101%**	**102%**
Remainders vs. Controls	**98%**	**99%**	**99%**	**98%**	**98%**

Assay Results- Children's Hospital Boston
Coefficients of Variation (CV)

	T-Chol	Trig	Glucose	IgG	Avg.
Reproducibility Study (few donors, many repeats)					
Cores	**4.4%**	**6.1%**	**6.6%**	**4.6%**	**5.4%**
Remainders	**1.9%**	**4.7**	**3.5**	**3.0**	**3.3**
Controls	**1.4%**	**2.3%**	**0.3%**	**2.2%**	**1.5%**

Next Steps

- Publish results ("*Biopreservation and Biobanking*")
- Add functionality to prototype design
- Build and test "Beta" next generation prototype
- Kick off product development leading to a product launch

Automated Frozen Sample Aliquotting System

CRYOXTRACT
AN ALLIED MINDS COMPANY

Jorge de Dios (jorge.dedios@alliedminds.com)
Dr. Vincent Chun

DRAPER LABORATORY
Charles Stark Draper Laboratory

Dale Larson (dlarson@draper.com)
Stephen Bellio
Keith Baldwin
Karen Johnson
Linda Maloney
Michael Viens
Daniel Harrjes

Harvard Medical School
Technology and Engineering Center

John Slusarz

Brown University

Helena Judge Ellis
Deborah Venturini