

ARCHIVING BIOSPECIMENS

(Things to think about)

Jeanette Buckholz, RN, MSN
Department of Environmental Health
Center for Environmental Genetics
University of Cincinnati

STORAGE OF BIOSAMPLES CONSIDERATIONS

- Mini-cryo vials for sample containment
- Storage boxes for mini-cryo vials
- Trays for storage boxes
- Freezers for storage

Mini-Cryo Vials

- **Size and shape of tube**
 - 1 ml-2ml
- **Material**
 - Polypropylene
- **Tops**
 - Screw or flip top
 - Color coding
- **Bottom**
 - Rounded or conical
 - Free standing

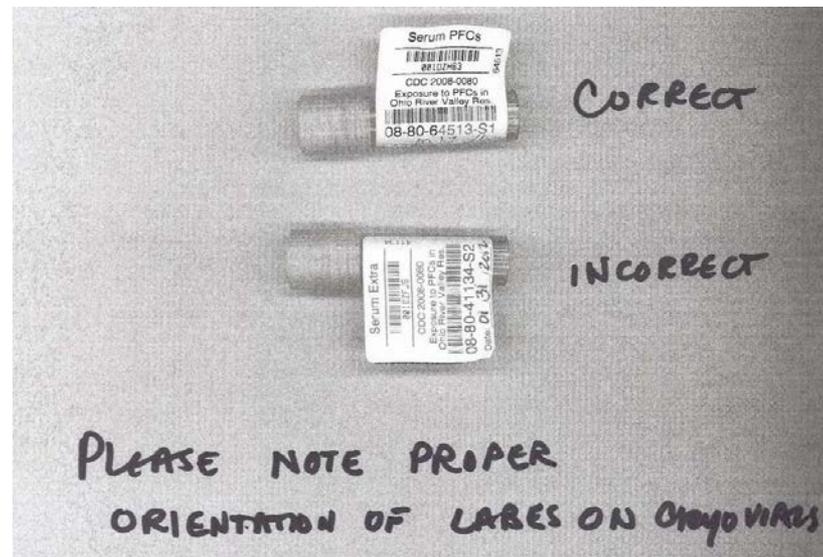


Labels

- **Labels**
 - Cryo-labels/ with cryopen
 - Pre-printed with bar code
- **Required information on biosample tube**
 - **Best practice:** Separate unique sample id for each sample that has a link in inventory database to identify sample.
 - Date of sample collection
 - Type of sample (serum, urine)

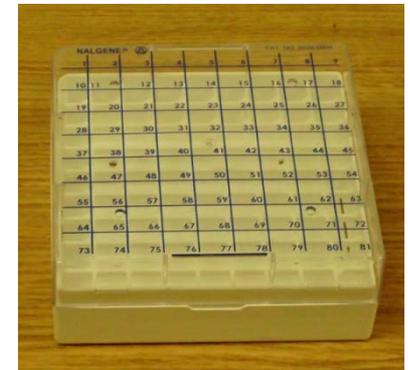


Correct placement of label



Storage Boxes

- **Type of box**
 - Plastic-long term storage
 - Cardboard-temporary storage
- **Size of box**
 - # of samples in box (ex 64, 81, 100)
 - Box Dimensions (ht, length, width)
- **Identification of box**
 - PI, Date, Study, number, etc
 - ID Should be on the bottom of the box
- **Benefits**

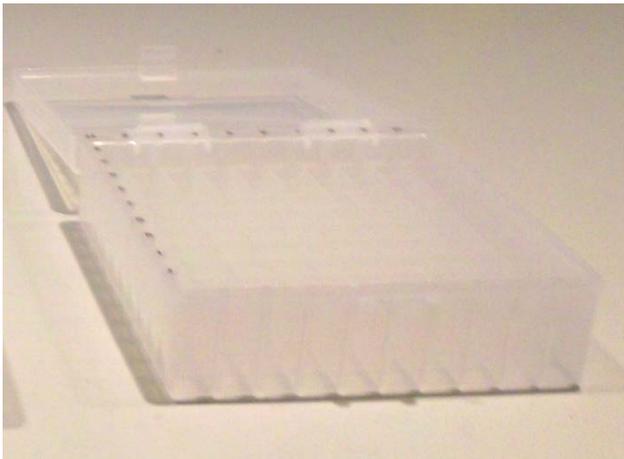
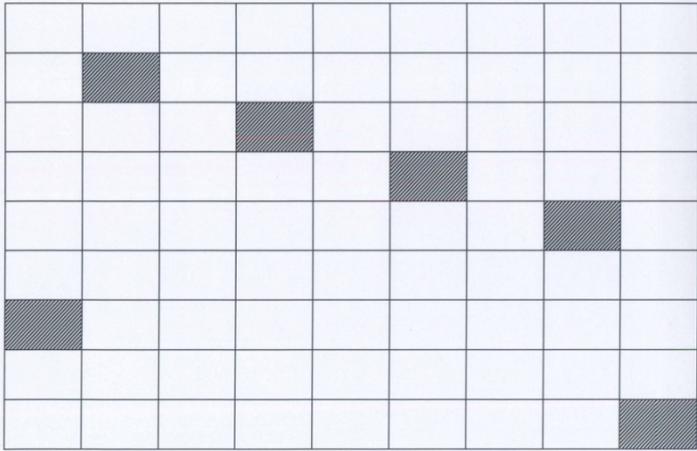




Box Maps

9 x 9 Box Number _____

Date: _____



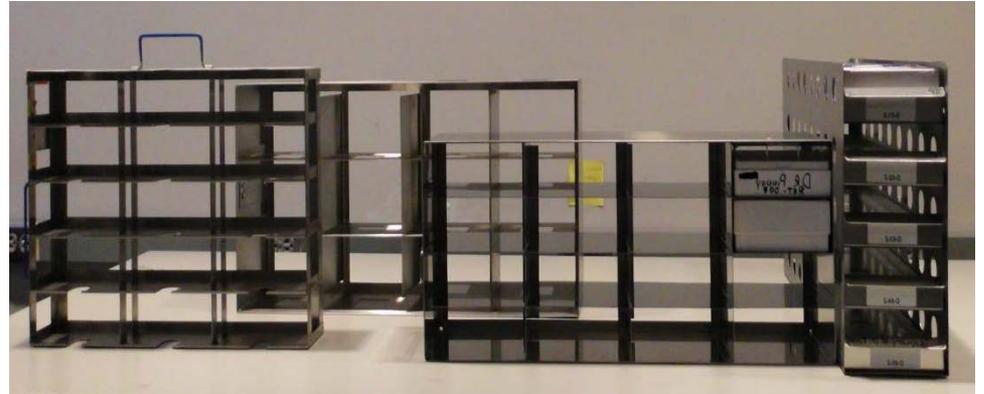
10 x 10 Box Number _____

Date _____



Trays

- Orientation
- Size
- Materials
- Labeling
- Benefits





D-01-2









11-16-96
TRANS #V
January 22, 1991
thru January
23, 1991

16-96
TRANS #AA
1/23/91
thru 1-24-91

18 1-24-91
thru 1-25-91

12-16-96
TRANS #AI
#34 1/30 thru 1/30/91

5V #
#32 1-23-91
1-24-91

12-16-96 TRANS #AE
#30 1-26-91 thru
1-27-91

12-16-96 TRANS #AK
#36 1-31-91 thru
1-31-91

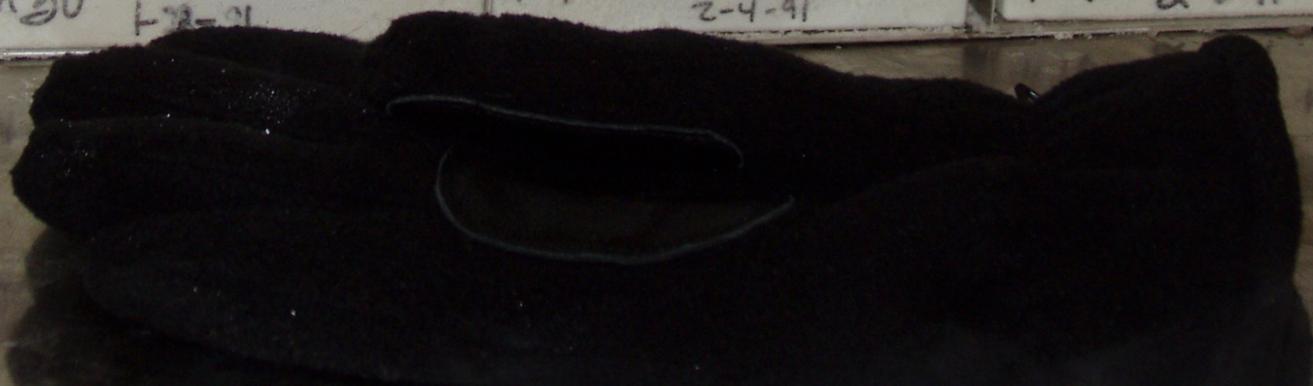
12-16-96 TRANS #AM
#38 2-1-91 thru
2-2-91

12-16-96 TRANS #AO
#40 2-4-91
thru
2-4-91

12-16-96 TRANS #AL
#42 2-5-91
thru 2-6-91

12-16-96 TRANS #AS
#44 2-7-91 thru
2-7-91

12-16-96 TRANS #AU
#46 2-8-91 thru
2-8-91



Freezers

- Size
- Shape
- Temperature
- Costs
 - Freezer maintenance
 - Back up
 - Replacement



Freezers Cont'd

Maintenance

- Room temperature control
- Scheduled preventative maintenance checks
- Local or central alarm system to monitor temperature
- Local temp recorder
- Fill it up and open as infrequently as possible

Back up

- Empty freezer with same shelf configuration
- CO2 Back up tank for compressor failure



Pulling the samples
(freezer diving)

**GOAL=GET IN AND OUT OF
THE FREEZERS WITH YOUR
SAMPLE(S) AS QUICKLY AS
POSSIBLE**

Preparation Before The Pull

- 75% of the work is done in preparation before you pull the samples.
- Identify the samples to be pulled according to research criteria.
- Create a “pull sample” spreadsheet with (freezer, shelf, tray, slot, date, and comments)
- Create an additional spreadsheet of alternate samples sorted by sample ID.

Pull and Alternate Sample Spreadsheets

Pull Sheet

	UC ID	Sample Type	Freezer No.	Shelf No.	Tray No.	Box No.	Slot No.	Date Distributed	Project Code	Year Drawn	PULL	Date Pulled	Comments
Control	66882	Plasma	2	1	43	ANS	9,5			1993	X		
Control	67593	Whole Blood	2	2	50	ATI	3,9			1993	X		
Control	65596	Whole Blood	3	3	129	ADQ	5,2			1992	X		
Case	65558	Whole Blood	3	4	134	ADG	6,4			1992	X		
Case	60848	Non-Buffered Urine	4	1	144	EX	6,7			1991	x		

Alternate Sample Sheet

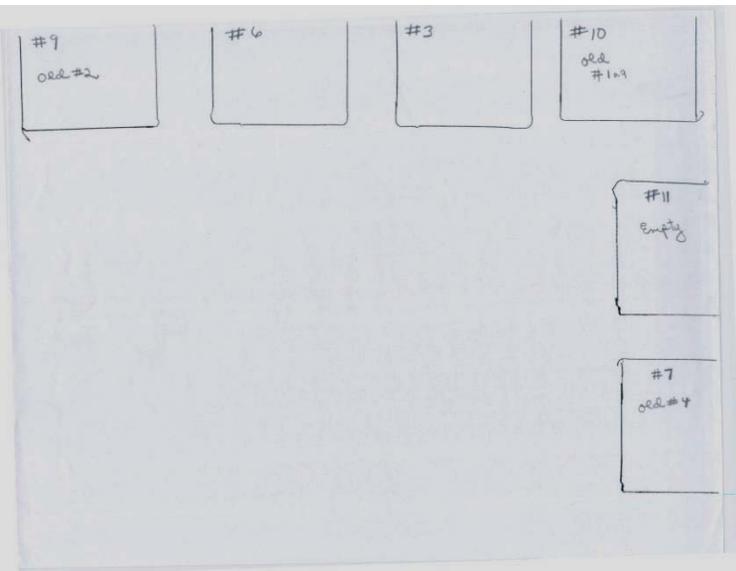
	UCID	Sample Type	Freezer No.	Shelf No.	Tray No.	Box No.	Slot No.	Date Distributed	Project Code	Year Drawn	PULL	Date Pulled	Comments
Case	60115	Whole Blood	4	2	148	D	7,4			1990			
Case	60115	Whole Blood	7	2	8	E	1,6			1990			
Case	60115	Serum	7	2	8	E	1,2			1990			
Case	60115	Serum	4	2	148	D	7,2			1990			
Case	60848	Whole Blood	4	1	144	EX	6,5			1991			
Case	60848	Whole Blood	7	2	11	EW	9,6			1991			
Case	60848	Serum	7	2	11	EW	9,4			1991			
Case	60848	Serum	4	1	144	EX	5,9			1991			

Preparation (con't)

- Identify your pull team (4 people-good to have one tall person)
- Team members must read the **Sample Pull Protocol**. If first time orient them with pictures before pull.
- Appropriate dress (shoes, long pants, sleeves, gloves)
- Determine date, time, & transportation for sample pull with team and confirm with freezer administration staff. (must stay for complete pull)
- Develop an “**attack plan**” to find the samples and share with the pull team.
- Attention to detail is very important.

Preparation con't “Attack Plan”

Freezer Map



Tray Map

Freezer # _____ 9 _____

43	44	45	46	47	48	49
50	51	52	53	54	55	56
57	58	59	60	61	62	63
64	65	66	67	68	69	70
71	72	73	74	75	76	77
179	182	183				

Preparation (Con't)

- Supplies needed for pull are gathered (sample box, blank box map, pens, labels, towel, tape, extra gloves, ruler, hemostat, cooler with dry ice)



Preparation (con't)



Pulling the Samples

- Have a detailed written protocol in which team members have an identified role.
- The following slides review our protocol.

Pulling the Samples

- One team member initiates the Sample Report Form.
- Work area is set up by other team members.



Report for FMMP Cyropreserved Biospecimen Removal

Project name:		
Date of sample pull:		
Members of FMMP sample pull team:		
	Name	Read Protocol?
Person #1		
Person #2		
Person #3		
Person #4 – UC staff member		
Arrival time in freezer room: _____ AM PM		
Temperature of Freezers at arrival		
Temp of Freezer #1		
Temp of Freezer #2		
Temp of Freezer #3		
Temp of Freezer #4		
Temp of Freezer #5		
Temp of Freezer #6		
Temp of Freezer #7		
Freezer alarm activation		
Alarm #1	Freezer	Time
Alarm #2	Freezer	Time
Alarm #3	Freezer	Time
As soon as an alarm goes off for a third time, the sample pull session is over. Third time = third ringing of a freezer alarm, regardless of the freezer.		
Incident #1		
Incident #2		
Departure time from freezer room: _____ AM PM		
Temperature of Freezers at departure		
Temp of Freezer #1		
Temp of Freezer #2		
Temp of Freezer #3		
Temp of Freezer #4		
Temp of Freezer #5		
Temp of Freezer #6		
Temp of Freezer #7		

Pulling the Samples

Team leader identifies the sample location from the Pull Sheet by:

- Freezer
- Shelf
- Tray
- Box
- Slot

And the team member pulls the tray from the freezer



Pulling the Samples

- Only one tray is carefully pulled at a time and placed on work area. Use a rolling cart that can be moved as needed.
- The Freezer door is immediately closed.



Pulling the Samples

- **Box** is pulled from tray and opened, box map removed.
- **Sample slot** is identified and sample pulled with a hemostat.
- **Sample ID & type** are read out loud and verified with the pull sheet by the other 2 staff members using the pull sheet.
- **Sample is crossed off the box map** with date, initials, study code; map is returned to box, placed in the tray and returned to the freezer.



Pulling the Samples

- The team leader records the date and comments on the pull sheet.
- If any problems identified, the team leader makes a note to follow-up. (Missing sample, wrong id, empty tube, sample not matching box map).



Pulling the Samples

- Team member takes the sample, and
- Verifies it again with pull sheet, and
- Places the sample in a transport box in a cooler with dry ice, and
- Writes the Sample ID, sample type & year in the location on the box map for the transport box.



Pulling the Samples

- These steps are repeated until all samples are pulled or when the team leader determines that the team is tired or the time frame to pull the samples is taking too long.
- Freezer face time for 100 samples is about 1-1 ½ hours –not including transportation to the freezer site.



Pulling the Samples

- Pulling samples is a very tedious job.
- Physically challenging –the freezers are cold (-80 C) and the trays are heavy. The room is noisy and drafty with a small space to work.
- Try to set a goal of how many samples to pull, but if the pull becomes frustrating, the pull team leader may choose to stop the pull and re-schedule. This will help to prevent problems.
- Usually a maximum of 100 – 125 samples can be pulled in a session.

After The Pull

- Samples that are pulled should be stored temporarily in a -80 freezer until ready to be distributed to the researcher or lab.
- Documentation of sample pull should be completed within one day of the sample pull.

Handwritten sample pull notes are transferred to the electronic pull sheet (Excel file).

FCU - Harley/Lu Lupus project Serum samples for controls subjects Pull sheet 28-Nov-12												✓ marked out on box maps ✓ code put in freezer database
UC_ID	Priority_Level	Sample_Type_Text	Freezer_no	Shelf_no	Tray_no	Box_no_oId	Box_no	Slot_no	Year_Drawn	Sample date closest to case Dx date	Pull date	Comments
✓ 66536	1A	Serum	2	1	45	1574 ✓	APC	3,5	1993	X	11-28-12	
✓ 67072	1A	Serum	2	1	45	1578 ✓	APG	6,1	1993	X	11-28-12	
✓ 67118	2	Serum	2	1	46	1588 ✓	APQ	1,2	1993	X	11-28-12	
✓ 67221	2	Serum	2	1	46	1608 ✓	AQK	4,8	1993	X	11-28-12	
✓ 67241	2	Serum	2	1	47	1612 ✓	AQO	4,7	1993	X	11-28-12	
✓ 67328	2	Serum	2	1	47	1630 ✓	ARG	1,1	1993	X	11-28-12	
✓ 67368	2	Serum	2	1	48	1638 ✓	ARO	1,1	1993	X	11-28-12	
✓ 67380	1	Serum	2	1	48	1640 ✓	ARQ	3,5	1993	X	11-28-12	
✓ 60142	1	Serum	2	3	59	1849 ✓	F-U	9,8	1994	X	11-28-12	replaced box map
✓ 60693	2	Serum	2	3	60	1857 ✓	FAA	5,3	1994	X	11-28-12	
✓ 60294	2	Serum	2	3	62	1964 ✓	FCM	7,3	1994	X	11-28-12	Box NOT in Temp (FCM) chamber No search for
✓ 65485	2	Serum	2	4	69	2118 ✓	FIM	3,3	1995	X	11-28-12	Replaced box map
✓ 66597	1	Serum	2	4	69	2141 ✓		6,3	1995	X	11-28-12	Replaced box map
✓ 67647	2	Serum	2	4	70	2219 ✓		2,1	1996	X	11-28-12	Replaced box map
✓ 62722	2	Serum	2	5	71	2227 ✓		9,1	1996	X	11-28-12	Replaced box map
✓ 67967	2	Serum	2	5	71	2229 ✓		4,3	1996	X	11-28-12	replaced box map
✓ 66089	2	Serum	2	5	72	2155 ✓		9,1	1995	X	11-28-12	Replaced box map
✓ 67217	1	Serum	2	5	72	2167 ✓		3,1	1996	X	11-28-12	replaced box map
✓ 66942	2	Serum	2	5	73	2181 ✓		7,3	1996	X	11-28-12	"

The sample distribution code and date is entered into the Freezer Inventory Database

ID#1:

Search

Specimens

Sample Type:	<input type="text" value="Whole Blood"/>	Date Distributed:	<input type="text"/>
Sample Date:	<input type="text" value="3/21/2008"/>	Project Code:	<input type="text"/>
Exam Year:	<input type="text" value="EXAM8"/>		
Freezer #:	<input type="text" value="7"/>	Year Transferred:	<input type="text"/> yyyy
Shelf #:	<input type="text" value="5"/>	<input type="checkbox"/> Sample Lost During Freezer Failure	
Tray #:	<input type="text" value="133"/>	<input type="checkbox"/> Participant Refused Sample Collection	
Box #:	<input type="text" value="2456"/>		
Old Box #:	<input type="text" value="BBBB"/>		
Slot #:	<input type="text" value="1,5"/>		

A report is run on the distribution code and a QC check is done to see that all samples have been entered into the inventory

FMMP Specimens used for Specific Project Code

Sample Count: 147

UC ID	Priority	Sample ID	Sample Type	Freezer	Shelf	Tray	Slot	Old Box #	New Box #	Drawn	Proj Code	Split/Return	From	Date Distrib
60102	1	160312	Serum	4	2	148	3,6	1	B	1990	HAR11	S		12/14/2011
60113	2	15176	Serum	4	2	148	4,7	3	D	1990	HAR11	S		11/28/2012
60142	1	15858	Serum	2	3	59	9,8	1849	F-U	1994	HAR11	S		11/28/2012
60153	2	16106	Serum	6	3	97	9,6	18	F-R	1994	HAR11	S		11/28/2012
60186	2	16896	Serum	7	2	8	3,5	18.1	S	1991	HAR11	S		11/28/2012
60294	2	19316	Serum	2	3	62	7,8	1963	FCM	1994	HAR11	S		11/28/2012
60311	1	160314	Serum	4	2	155	3,6	43	AR	1991	HAR11	S		12/14/2011
	1	19668	Serum	20			2,7	43	RET005	1991	HAR11			12/14/2011
60315	1	19750	Serum	3	5	163	3,7		07-043	2007	HAR11	S		11/28/2012
60344	2	20390	Serum	7	2	10	1,1	50	AY	1991	HAR11	S		11/28/2012
60367	2	20852	Serum	7	2	10	4,7	54	BC	1991	HAR11	S		11/28/2012
60398	1A	21523	Serum	7	2	10	5,9	60	BI	1991	HAR11	S		11/28/2012
60479	2	23183	Serum	3	1	118	1,1	77	BZ	1991	HAR11	S		11/28/2012
60542	1	24481	Serum	3	1	118	4,7	89	CL	1991	HAR11	S		11/28/2012
60546	2	24570	Serum	7	2	12	3,5	90	CM	1991	HAR11	S		11/28/2012
60554	1A	160321	Serum	7	2	12	1,2	92	CO	1991	HAR11	S		12/14/2011
	1A	24733	Serum	20			3,3	92	RET005	1991	HAR11			12/14/2011
60568	2	25029	Serum	7	2	12	5,9	94	CQ	1991	HAR11	S		11/28/2012
60624	2	26215	Serum	4	2	154	5,9	105	DB	1991	HAR11	S		11/28/2012
60650	2	26782	Serum	4	2	154	1,2	111	DH	1991	HAR11	S		11/28/2012
60693	2	27722	Serum	2	3	60	5,3	1857	FAA	1994	HAR11	S		11/28/2012
60695	2	27764	Serum	7	2	14	2,3	120	DQ	1991	HAR11	S		11/28/2012
60697	2	160306	Serum	3	5	166	1,4	07-075		2007	HAR11	S		12/14/2011
	2	27802	Serum	20			2,1		RET005	2007	HAR11			12/14/2011
60757	2	29103	Serum	6	4	101	7,3	1929	FBL	1994	HAR11	S		11/28/2012
60799	2	160320	Serum	7	2	14	3,6	142	EM	1991	HAR11	S		12/14/2011
	2	30039	Serum	20			3,5	142	RET005	1991	HAR11			12/14/2011

After the pull Splitting a sample

Because samples are so valuable, if a researcher or lab needs less volume, a sample can be split from the main stock.

- Preparation is the key to splitting the samples.
- Defrost samples in refrigerator slowly overnight. This decreases degradation and fragmentation of proteins (DNA).

Thawing a Sample

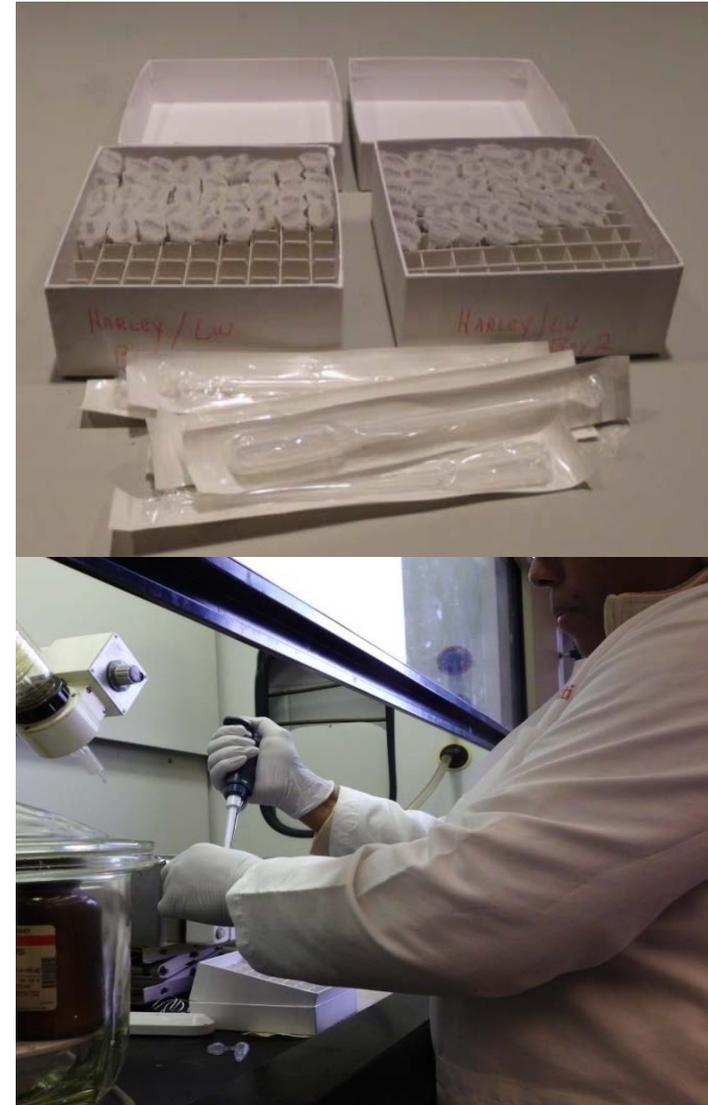
The best way to thaw a sample is to:

- Move sample from -80 to -20 for 24 hours.
- Move sample from -20 to -3 for 24 hours.
- Gently use a Vortex Mixer to evenly distribute sample contents. (serum, plasma, urine)
- To refreeze sample—reverse thawing process.



Splitting a Sample

- Set up boxes so original stock is in the same position as the new vial.
- Pre-label the new vial.
- Have a second person check the ID on the pre-labeled vial before starting.
- Transfer under a hood.
- Use gloves and a new pipette for each sample.
- Copy the box map for the new box.



Prepare samples for Researcher or lab

- Make a copy of the box map for your records
- Label the box for the researcher
- Prepare sample transfer record (Manifest)
(Manifest) should include
 - sample ID
 - date
 - amount
 - type of sample
 - location in box
 - date sent or transferred.

Sample Transfer

	66536	67072	67118	67221	67241	67322	67368	67380
	S	S	S	S	S	S	S	S
	1993	1993	1993	1993	1993	1993	1993	1993
60172	60693	65485	66597	67647	68722	67967	66089	67217
S	S	S	S	S	S	S	S	S
1994	1994	1995	1995	1996	1996	1996	1995	1996
66942	62448	61533		60479	60542	66653	64906	64966
S	S*	S		S	S	S	S	S
1996	1992	1991		1991	1991	1993	1992	1992
65635	65692	65789	65069	65114	65565	65816	64603	60315
S	S	S	S	S	S	S	S	S
1992	1992	1992	1992	1992	1992	1992	1992	2007
61327	60837	61422	60113	61892	61615		60624	60650
S	S	S	S	S	S		S	S
2007	1991	1991	1990	1991	1991		1991	1991
68505	67175	67293	67521	67777	67972	62770	65758	60153
S	S	S	S	S	S	S	S	S
2008	1993	1993	1993	1993	1993	1995	1995	1994
62448	61220	60757	64992	65938		61624	64319	66585
S*	S	S	S	S		S	S	S
1995	1994	1994	1995	1995		1996	1998	1996
67134	67189	66354	65976	66028	66071	60186	60344	60367
S	S	S	S	S	S	S	S	S
1996	1996	1992	1992	1992	1992	1991	1991	1991
60398	60546	60568	60695		62176	61968	61474	61154
S	S	S	S		S	S	S	S
1991	1991	1991	1991		1991	1991	1991	1991

FERNALD COMMUNITY COHORT

Fernald Community Cohort					
Biospecimen Material Transfer Form for Harley/Lu lupus project #62					
x <input type="checkbox"/> in the FCC to Dr. Judith James Oklahoma Medical Research Foundation					
Specimens transferred from _____ back to the FCC					
Date of sample transfer: 6-Dec-12					
Signature of FCC personnel:				Jenny Buckholz	
Signature of investigator or representative:					
RID	Sample Type	Year Drawn	Box	Slot	Date Transferred
108449	Serum	1993	1	1,2	12/6/2012
105791	Serum	1993	1	1,3	12/6/2012
102119	Serum	1993	1	1,4	12/6/2012
109570	Serum	1993	1	1,5	12/6/2012
100749	Serum	1993	1	1,6	12/6/2012
102114	Serum	1993	1	1,7	12/6/2012
102304	Serum	1993	1	1,8	12/6/2012
100196	Serum	1993	1	1,9	12/6/2012
105929	Serum	1994	1	2,1	12/6/2012
104016	Serum	1994	1	2,2	12/6/2012
105822	Serum	1995	1	2,3	12/6/2012
108924	Serum	1995	1	2,4	12/6/2012
102473	Serum	1996	1	2,5	12/6/2012
104784	Serum	1996	1	2,6	12/6/2012
103160	Serum	1996	1	2,7	12/6/2012
100178	Serum	1995	1	2,8	12/6/2012
109536	Serum	1996	1	2,9	12/6/2012
101289	Serum	1996	1	3,1	12/6/2012
105833*	Serum	1998	1	3,2	12/6/2012
105760	Serum	1991	1	3,3	12/6/2012
105056	Serum	1991	1	3,5	12/6/2012
107913	Serum	1991	1	3,6	12/6/2012
109552	Serum	1993	1	3,7	12/6/2012
105777	Serum	1992	1	3,8	12/6/2012
100718	Serum	1992	1	3,9	12/6/2012
101533	Serum	1992	1	4,1	12/6/2012
106972	Serum	1992	1	4,2	12/6/2012
107122	Serum	1992	1	4,3	12/6/2012
105397	Serum	1992	1	4,4	12/6/2012
102056	Serum	1992	1	4,5	12/6/2012

Shipping Biological Samples

REGULATIONS AND GUIDELINES for SHIPPING

Carrier such as FedEx

DOT-Department of Transportation

IATA-International Air Transport Association

ICAO-International Civil Aviation Administration

CDC-Center for Disease Control

TSA-Transportation Safety Administration

FDA- Food and Drug Administration

OSHA-Occupational Safety and Health Administration

FAA-Federal Aviation Administration

Categories of Biological Substances for the Purpose of Shipping

- CATEGORY A Infectious Substances
- CATEGORY B Biological Substances
- EXEMPT Human or Animal Specimens – sent for a clinical tests or environmental markers.
- Non-regulated Biological Materials – Food.

PREPARING THE SAMPLES FOR SHIPMENT

THINGS TO CONSIDER:

- Transport by air or ground
- Regulations by carriers-check shipping & labeling protocols for FedEx, UPS, CDC, etc.
- Receiving entity requirements.
- Transport with (water) ice, gel packs or dry ice or both.
- Time of year (season)
- Day of week (never on Friday)
- Supplies and labels needed (Commercial shipping kits are available for purchase, but boxes can be reused).

BASIC PACKAGING

- The sample must be placed in a leak-proof primary receptacle (vial). Multiple primary receptacles must be individually wrapped or separated to prevent contact (sample box).
- Cardboard boxes can be used for shipping.
- Secure with string to prevent from opening (rubber bands will break with dry ice in shipping container)
- Place in outer package with suitable cushioning material.



Considerations for Shipping Refrigerated Materials

- Gel coolants are preferable to wet ice or ice packs.
- Pre-cool insulated container, if possible.
- Arrange products inside the insulated container, allowing space for coolants.
- Place sufficient number of coolants on top and around the product.
- Fill all void space to prevent movement.
- Place insulated container inside a corrugated outer box and seal with packing tape.

Consideration for Shipping Frozen Materials

DRY ICE

- Dry Ice Facts
 - Dry ice is solidified carbon dioxide
 - It is extremely cold, -109.3 F (-79 C)
 - Does not melt-changes directly from solid to gas
 - Solid block or pellets
- Safe Handling of Dry Ice
 - Avoid contact with skin and eyes. Never handle dry ice with bare hands. Can cause severe frostbite in seconds.



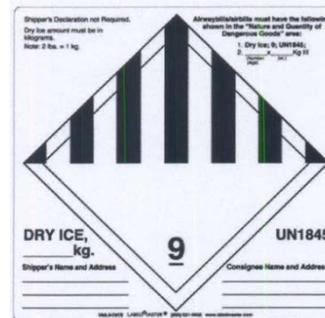
Packing with Dry Ice

- Pre-cool the insulated container if possible.
- Insulated container should be at least 1.5 inches thick.
- When arranging materials inside the insulated container, allow enough space for the dry ice.
- Don't use large chunks of dry ice.
- Place sufficient amount of dry ice on top and around the materials. (1-2 kilograms (2-4 lbs) of dry ice per 24 hours). Should consider enough for 2 days. Adding a gel pack will help to increase the time.
- Fill void space with packing material (peanuts, etc) to prevent movement.



Packing with Dry Ice

- If a plastic bag is used close it, but do not completely seal it, the carbon dioxide gas created by the dry ice must be allowed to vent. Place lid on the insulated container. Place the insulated container inside an outer corrugated box.
- Close and securely tape the box with packing tape, but careful not to completely seal it. Address of sender and receiver should be clearly visible.
- Follow dry ice shipping regulations, record amount of dry ice used on label (UN1845) and airbill.



Make Sure it Gets There

- Send as FedEx Express priority overnight for next morning delivery.
- Obtain call confirmation number and tracking number.
- Make sure it is picked-up.
- Notify receiving entity of shipment and tracking number.
- Next day, be sure to check tracking number to verify that shipment has been received.

Questions?

- Jeanette Buckholz, RN, MSN
- buckhojm@ucmail.uc.edu
- 513-558-0487

Acknowledgment

- This presentation was prepared by the Integrated Health Sciences Core of the University of Cincinnati Center for Environmental Genetics, supported by funding from the National Institute of Environmental Health Sciences (P30 ES 006096).