CRYOPRESERVATION OF WHOLE BLOOD SAMPLES COLLECTED IN THE FIELD FOR A LARGE, PROSPECTIVE COHORT STUDY.

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ABSTRACT

Cryopreserved lymphocytes are an extremely valuable resource for epidemiologic studies because they can be used to measure a variety of cellular functions and biomarkers and to provide an infinite source of DNA. Successful cryopreservation requires addition of a cryoprotective agent such as glycerol or dimethyl sulfoxide (DMSO) to the blood followed by freezing at a controlled rate to ultralow temperatures. The requirement for specialized and expensive equipment for cryopreservation has inhibited the collection of blood for this purpose by studies in which biospecimens are obtained in non-laboratory settings. In this study, we developed a protocol to allow us to successfully cryopreserve whole blood samples collected in the field from enrollees in the Cancer Prevention-3 (CPS-3) cohort study without using specialized cryopreservation equipment. Blood was collected in a sodium heparin-containing vacutainer at six outdoor fund-raising events and transported on an ice-water bath via courier to one of four different processing labs. The blood was then transferred to a cryovial containing 10% DMSO, placed in an enclosed Styrofoam rack, and frozen to -80°C for a minimum of 1 day. The samples were then shipped on dry ice to a biorepository, where they were placed in liquid nitrogen tanks for long term storage.
To evaluate the effectiveness of the protocol, 30 samples were tested for their viability, lymphocyte yield, and ability to be transformed by EBV. Although lymphocyte recovery varied considerably, all samples yielded at least 2 x 10^6 cells and had high viability (≥86%). All samples were successfully transformed by EBV and yielded immortalized cell lines within 15 days of treatment with the virus. These findings indicate that whole blood samples collected in the field can be successfully cryopreserved without using specialized equipment.

BACKGROUND

Requirements for Cryopreservation of Cells
- Addition of cryoprotective agent (e.g. DMSO)
- Slow freezing to -50° C
- Long-term storage at < 130ºC

Previous Studies
- Viability/transformability of cryopreserved lymphocytes or whole blood is similar to fresh samples
- Transformability not affected by extended storage in liquid nitrogen
- Lymphocytes from blood collected in sodium heparin have been successfully transformed

Cancer Prevention Study-3 (CPS-3)
- Prospective cohort study
- Pilot enrollment began in 2006
  - 6 collection sites
  - 4 processing labs (Quest Diagnostics)
  - 1 biorepository (Fisher)

RESEARCH QUESTIONS

Can whole blood cryopreservation be done successfully at a number of labs?
- Blood processors have no specialized training
- Controlled rate freezers not available

Does transport between blood collection and processing effect cryopreservation?
- Temperature variation
- Time differences

Does shipping to the biorepository after processing effect cryopreservation?
- Time differences

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METHODS

10 Combinations of handling variables
- Transport temperature
- Time to freezing
- Time from freezing to liquid nitrogen

Use 3 independent samples for each combination (n=30)

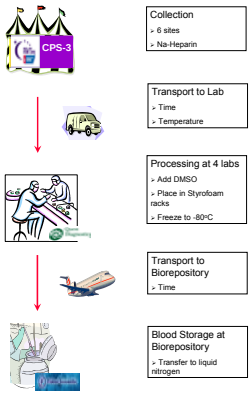
Varied by:
- **Collection Site**
  - Texas 1
  - Texas 2
  - Georgia 1
  - Georgia 2
  - California 1
  - California 2
- **Processing Lab**
  - Atlanta
  - Dallas
  - Los Angeles
  - San Jose

Collection to Processing Variables

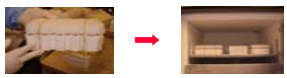
Processing Time [1]	Short			Long		
Time to LN Storage [2]	Short	Moderate	Long	Short	Moderate	Long
Temperature [3]						
<40 °F	California 2, Short	Georgia 1, Short	California 1	California 2, Long	Georgia 1, Long	
40-42 °F		Georgia 2, Short	Texas 1, Short		Georgia 2, Long	Texas 1, Long
48+ °F						Texas 2

[1] Time from blood draw to processing at Quest Diagnostics.
[2] Time from Quest Diagnostics to the time when the samples were placed in liquid nitrogen at Fisher BioServices.
[3] Temperature of samples before processing at Quest Diagnostics.

Blood Handling Variables



Freezing at a Controlled Rate- "Poor Man's Freezer"

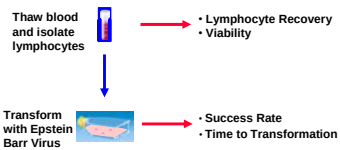


Enclose cryovials in a Styrofoam rack

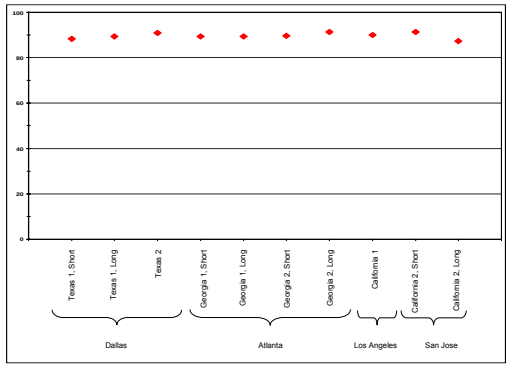
Freeze in -80ºC freezer for at least 1 day

RESULTS

Lymphocyte Isolation and Transformation (ATCC)



Minimal Lab and Site Variation for Viability



Effects of Blood Handling Procedures

Collection Site, Processing Lab	Transport Temp (°F)	Time to Processing (hours)	Time to Liquid N2 (hours)	Viability (%)	Time to Transform (days)
Texas 1, Short Dallas	40	13.9	74.1	89.0 ± 2.9	10.0
Texas 1, Long Dallas	40	8.3	74.3 ± 0.1	88.3 ± 1.5	10.0
Texas 2 Dallas	55	13.4	70.1	91.0 ± 2.0	12.0 ± 1.7
Georgia 1, Short Atlanta	37	3.9	84.4 ± 0.2	89.3 ± 1.5	11.0 ± 1.7
Georgia 1, Long Atlanta	37	6.1	84.7 ± 0.1	89.0 ± 1.7	14.3 ± 2.3
Georgia 2, Short Atlanta	49	5.5	76.8 ± 1.3	89.7 ± 1.5	10.0
Georgia 2, Long Atlanta	39	8.2 ± 0.1	80.1 ± 3.1	91.3 ± 1.5	11.0 ± 1.7
California 1 Los Angeles	40	4.9	256.2	90.0 ± 2.0	12.0 ± 1.7
California 2, Short San Jose	39	4.9	62.7	91.3 ± 0.6	9.7 ± 1.5
California 2, Long San Jose	39	8.3	62.8	87.3 ± 1.5	11.0 ± 1.7

CONCLUSIONS

Lymphocytes were successfully immortalized using the CPS-3 collection protocol
- Blood collected in sodium heparin vacutainers
- Graduated freezing using "Poor Man's Freezer" method
- Transport temperature: 37-55ºF
- Transport time: 3.9-13.9 hours
- Time from freezing to liquid nitrogen: 62-256 hours

There was no significant site or lab variation in the quality of the cryopreserved whole blood samples