CTRNet Standard Operating Procedure Blood Derivatives – Extraction of DNA			
SOP Number:	08.02.004	Version:	e2.0
Supersedes:	8.2.004 e1.0	Category:	Material Handling and Documentation - Blood

Approved By:	CTRNet Management Group (CMG)	01-June-2012
	Per: Brent Schacter	13-June-2012

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour biobank program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing deoxyribonucleic acid (DNA) from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for CTRNet biobanks to follow when extracting DNA from blood samples.

2.0 SCOPE

This standard operating procedure (SOP) describes how DNA should be extracted from blood samples. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

Note: When adopting this SOP for local use please reference CTRNet.

3.1 *CTRNet Policy: POL 5 Records Documentation*
3.2 *CTRNet Policy: POL 2 Ethics*
3.3 *CTRNet Policy: POL 4 Privacy and Security*
3.4 *CTRNet Policy: POL 7 Material Information Handling*
3.5 *CTRNet Standard Operating Procedure: SOP 08.02.001 Blood Collection*
3.6 *CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management*

4.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from CTRNet member biobanks who are responsible for extracting DNA from blood.

Tumour Biobank Personnel	**Responsibility/Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting DNA from blood samples.

5.0 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

The materials, reagents, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure

Materials and Equipment	Materials and Equipment (Site Specific)
Markers, ink and pens	
Appropriate labels for tubes and vials	
Tube of previously isolated Buffy Coat from blood sample	
Biohazardous waste container and autoclave bags	
2 ml Microfuge tubes	
1.5 ml centrifuge tubes	
2 ml cryotubes	
Racks for microfuge tubes	
Tube racks for water bath	
Vortex mixer	
Microcentrifuge	
Pipettes	
Sterile pipette tips with aerosol barrier	
Micropipettors	
Transfer pipettes	
Isopropanol	
DNA Extraction Kit	
A shaking Heat Block like the Eppendorf Thermomixer or normal heating block or water bath.	
-80° C and -20° freezer	
Storage boxes	
Disposable gloves	
Hot water bath (set at 55° C)	
Tube racks for water bath	
Rolling rack (rotator mixer)	
95% ethanol	
70% ethanol	
Refrigerator at 4° C	

6.0 DEFINITIONS

See the CTRNet Program Glossary: http://www.ctrnet.ca/glossary

7.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from blood samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results. Work with tubes in the same order for the duration of the procedure to minimize the potential for sample mix up.

7.1 Extraction of DNA from Blood Samples Using a Column Based Kit

7.1.1 Document the method of DNA extraction. There are several commercially available DNA extraction kits available, follow the detailed procedure outlined in the appropriate commercial kit handbook.

7.1.2 Treat all blood as potentially infectious.

7.1.3 DNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the tumour biobank.

7.1.4 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.

7.1.5 Thaw the previously frozen Buffy Coat by gentle agitation in a 37º C water bath.

7.1.6 Keep the thawed tube on ice until starting the extraction procedure.

7.1.7 Use a DNA extraction kit, follow protocols, and document

7.1.8 Genomic DNA can be stored at 4ºC.

7.1.9 Quantitate DNA by spectophotometry and/or fluorometry. Record concentration on stock tube.

7.1.10 DNA is a weak acid and at 4ºC may be subject to acid hydrolysis. For long-term storage keep DNA at -80º C. Avoid subjecting the DNA to freeze/thaw cycles to prevent fragmentation of the genomic DNA.

7.1.11 Place DNA in storage boxes and record storage location.

7.2 Data Elements to Track

7.2.1 Source sample ID and type (whole blood, buffy coat, etc.) and volume

7.2.2 DNA sample Id.

7.2.3 Date extracted

7.2.4 DNA extraction method

7.2.5 DNA volume

7.2.6 DNA concentration and quantitation method

7.2.7 Ratio of A260nm/A280nm

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Declaration of Helsinki
http://www.wma.net/en/30publications/10policies/b3/index.html

8.2 Tri-Council Policy Statement 2; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, December 2010.

http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/

8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420

8.4 Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories

8.5 US National Biospecimen Network Blueprint
http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

8.6 SOP #: BIO-SOP-BLD-PRO-DNA. Blood Sample Processing November 20, 2006 Procure, Quebec Prostate Cancer Biobank

9.0 APPENDICES

9.1 Appendix A **–** Preparation of Buffers and Reagents Required for DNA Extraction.

10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
LP 001.001	2005	JdSH	CTRNet Generic SOP for Blood Collection and Processing
8.2.004	Jan 2008	JdSH	Revised to cover only extraction of DNA from blood cells
8.2.004	April 2009	JdSH	Removed the alternate freezing without isopentane Updated version to e1.1 (minor edits)
8.2.004		TS	Phenol Chloroform and Column-based methods have been separated into 2 SOPs. See 8.2.005. Renamed: DNA Extraction from blood-Column based method Removed supplies etc relevant to phenol/chloroform DNA extraction method Removed section for steps for phenol/chloroform DNA extraction method Added section for: Data elements to track.
8.2.004 e1.1	June 2012	CMG	Grammatical and formatting throughout Definitions removed Revision History moved to bottom Reference links updates Updated SOP references Section 5.0: Added “biohazardous…” and removed many materials/equipment items. Section 7.0: Added “work with tubes…” to paragraph. Revised title 7.1 using “a column based” kit. Deleted first paragraph in 7.1. Added 7.1.8 “Quantitate DNA…”

			Deleted 7.2. Created new 7.2 “Data Elements to Track” • Deleted the reference to “Qiagen Flexigene DNA Kit Handbook”

PREPARATION OF BUFFERS AND REAGENTS REQUIRED FOR DNA EXTRACTION

NOTE: Suppliers and Brands can be substituted with other appropriate brands.

Buffer A (for 500 mL):
10mM TRIS pH 7,9
2 mM EDTA pH 8
40mM NaCl

Proteinase K:
20 units/mg (Invitrogen # 25530-015)
Re-suspend in 10mL of storage solution
Dilution = 10mg/mL

Storage solution for proteinase K (for 50mL):
10 mM TRIS pH7,5
20 mM $CaCl_2$
50% Glycerol (Life Technologies cat# 25530-015)

TRIS saturated phenol:
- Put at 55°C to liquefy the crystal phenol.
- Add 0,1% of 8-hydroxyquinolin ⇒ RNAse inhibitor
- Mix
- Add an equal volume of TRIS 1M pH8
- Mix 30 minutes
- Let stand until the phases separate (3 hours to overnight, at 4°C)
- Take out the supernatant
- Add 500 mL TRIS 0,1M pH8
- Repeat until the phenolic phase pH is > 7.6
- Store at 4°C in a dark bottle

Phenol/Chloroform/Iso: (Keep at 4°C in a dark bottle)
Mix in following ratio- 25:24:1)
For 200 ml use
Phenol – 100 ml
Chloroform – 96 ml
Isoamyl Alcohol – 4 ml

Chloroform/Iso: (Keep at room temperature in a dark bottle)
Ratio (24:1)
For 200 ml solution use 192 ml Chloroform and 8 ml Isoamyl Alcohol.

TE buffer:
10mM TRIS pH 7,6
1mM EDTA pH 8