CTRNet Standard Operating Procedure DNA Extraction from Blood			
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Approved By:	CTRNet Management Group (CMG)	01-June-2012
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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 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 white blood cells obtained from 5 to 10 mls of whole blood samples (EDTA or ACD) using the phenol/chloroform (organic solvent) method.

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 and Documentation*
3.2 *CTRNet Policy: POL 2 Ethics*
3.3 *CTRNet Policy: POL 4 Privacy and Security*
3.4 *CTRNet Policy: POL 7 Material and 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 labelling 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 white blood cells from 5-10 mls of whole blood sample	
14 ml polypropylene tube	
1.5 ml centrifuge tubes	
2 ml cryovial	
Tranfer pipette (3 ml)	
Racks for 1.5 ml centrifuge tubes	
Centrifuge for 14 ml tubes	
Racks for 14 ml tubes	
Microcentrifuge	
Sterile pipette tips with aerosol barrier	
Micropipettors	
-80º C and -20º C freezer	
Storage Boxes	
Disposable gloves	
Water bath (set at 60° C)	
Tube racks for water bath for 14 and 2 ml tubes	
Rolling rack to mix purified DNA	
10 ml glass pipettes for transferring phenol and chloroform (do not use polystyrene)	
Pipette gun for 10 ml glass pipettes	
95% ethanol - ice cold	
70% ethanol - ice cold	
Lysis solution*	
Proteinase K 20 mg/ml	
TRIS Buffer saturated Phenol	
Chloroform /isoamyl alcohol*	
5 M NaCl	
TRIS EDTA (TE) Buffer pH 8.0	
Refrigerator at 4° C	

* See Appendix A – Preparation of Buffers and Reagents required for DNA Extraction.

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.

7.1 Extraction of DNA from Blood Samples using the Phenol/Chloroform Method

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the sample. It is possible to keep the supernatant at 4º C between each step. For buffer recipes see Appendix A.

7.1.1 Treat all blood as potentially infectious.

7.1.2 Phenol and chloroform are organic solvents and must be used in a fume hood. All phenol and chloroform waste must be discarded into the appropriate waste container for organic solvents.

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

7.1.4 Re-suspend white cell pellet in 2 ml of Lysis Buffer using a 3 ml transfer pipette.

7.1.5 Using the same transfer pipette, transfer cells and lysis buffer to a 14 ml polypropylene screw cap tube. If pellet is smaller or larger than usual adjust volume of lysis buffer accordingly.

7.1.6 Add 20 mg/ul Proteinase K to a final concentration of 200 ug/ml. Pipette up and down to mix using a 3 ml transfer pipette. To avoid shearing the genomic DNA do not vortex to mix.

7.1.7 Incubate 2-4 hours or overnight in a water bath at 60º C. Mix tube every 30 minutes when possible.

7.1.8 Add an equal volume of TRIS saturated Phenol.

7.1.9 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

7.1.10 Centrifuge at 900 g for 5 minutes at room temperature.

7.1.11 Label a new 14 ml tube.

7.1.12 Using a 1 ml pipettor, transfer upper phase (aqueous layer containing DNA) to the new 14 ml tube being careful not to remove the milky layer of the interphase.

7.1.13 Add an equal volume of 50:50 phenol:chloroform/isoamyl alcohol.

7.1.14 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

7.1.15 Centrifuge at 900 g for 5 minutes at room temperature.

7.1.16 Label a new 14 ml tube.

7.1.17 Using a 1 ml pipettor, transfer upper phase to the new 14 ml tube being careful not to remove the milky layer of the interphase.

7.1.18 Repeat steps 13-17 for the second 50:50 phenol:chloroform/isoamyl alcohol extraction.

7.1.19 Repeat steps 13-17 a third time if the interface is still very thick and milky.

7.1.20 Add an equal volume chloroform/isoamyl alcohol.

7.1.21 Mix tube by rocking back and forth approximately 70 strokes per minute for at least 2 minutes.

7.1.22 Centrifuge at 900 g for 5 minutes at room temperature.

7.1.23 Label a new 14 ml tube.

7.1.24 Using a 1 ml pipettor, transfer upper phase to the new 14 ml tube. There should be little to no milky layer at the interphase.

7.1.25 Make the aqueous purified DNA 1M by using 5M NaCl. Mix gently.

7.1.26 Add 2.5 times the volume of ice cold 95% ethanol (stock kept at -20º C) and rock tube gently back and forth to precipitate out DNA.

7.1.27 Using a 1 ml pipette tip or 3 ml transfer pipette, transfer the DNA to a 1.5 ml microfuge tube.

7.1.28 Centrifuge at 180000 x g for 1 minute in a microfuge at room temperature.

7.1.29 Discard supernatant.

7.1.30 Add 500 ul of ice cold 70% ethanol and flick tube until DNA pellet dislodges from bottom of tube.

7.1.31 Centrifuge at 180000 x g for 1 min. in a microfuge at room temperature.

7.1.32 Discard supernatant.

7.1.33 Centrifuge at 180000 x g for 1 min. in a microfuge at room temperature. Discard supernatant.

7.1.34 Re-suspend DNA pellet in 500ul TE buffer. Adjust volume of TE buffer depending on the size of the DNA pellet.

7.1.35 Transfer DNA to a 2.0 ml cryovial for long term storage.

7.1.36 To ensure DNA is uniformly dissolved; Incubate at in 60 º C water bath and/or rotate overnight.

7.1.37 Store DNA at -80º C.

7.1.38 Discard all phenol and chloroform waste into the appropriate waste container for organic solvents.

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
8.2.005 e1.0	June 2011	TS	Phenol Chloroform and Column-based methods have been separated into 2 SOPs. *See SOP 08.02.004 Blood Derivatives – Extraction of DNA*.
8.2.005 e1.0	June 2012	CMG	• Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references

PREPARATION OF BUFFERS AND REAGENTS REQUIRED FOR DNA EXTRACTION:

Lysis Buffer: 5% Sarkosyl
10 mM TRIS-HCl pH 8.0
10 mM EDTA pH 8
75 mM NaCl

Chloroform/Isoamyl Alcohol : Keep at Room Temperature in a dark bottle in a flammable storage cabinet. Ratio (28:1)