CTRNet Standard Operating Procedure RNA Extraction from Blood Samples			
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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 001.001	2005	JdSH	CTRNet Generic SOP for Blood Collection and Processing
8.2.003	2008	JdSH	Revised to cover only extraction of RNA from blood cells

1.0 PURPOSE

Genomic studies often utilize nucleic acids (DNA and RNA) derived from patient samples. When extracting and storing RNA from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. RNA degradation is a major problem during the collection, processing, and storage of clinical samples. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow when extracting RNA from blood samples.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how RNA 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 POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet SOP 8.2.001 Blood Collection
6. CTRNet SOP 8.2.002 Blood Processing and Storage
7. CTRNet SOP 8.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories that are responsible for extracting RNA from blood.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Responsible for labeling tubes and extracting RNA from blood samples.	

5.0 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

The materials, 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	
1 PAXgene Blood RNA tube (full)	
Qiagen RNA extraction Kit	
2 Cryotubes	
Ethanol (100%) pure grade	
Pipettes	
Sterile, aerosol-barrier, RNase-free pipette tips	
Vortex Mixer	
Variable speed Microcentrifuge (1000-8000 x g capacity) that can hold 2 ml microfuge tubes	
Centrifuge with ability to reach 3000-5000g equipped with swing bucket rotor to hold PAXgene blood RNA tubes	
A shaking Heat Block like the Eppendorf Thermomixer or normal heating block or water bath.	

Disposable Gloves	
Storage Boxes	
RNase inhibitor to wipe down bench (such as RNaseZap from Ambion)	

6.0 DEFINITIONS

Buffy Coat: A thin grayish white layer of white blood cells (leukocytes and platelets) found covering the top of packed erythrocytes (red blood cells) of a hematocrit.

RNA: A molecule that conveys the genetic information derived from DNA to the ribosomes within cells.

Ribonucleases (RNases): These are very stable and active enzymes that usually do not require cofactors to function. They are difficult to inactivate and even minute amounts are sufficient to degrade RNA.

7.0 PROCEDURES

This procedure is intended to ensure that RNA 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 RNA from blood samples – general extraction considerations

Avoiding Cross Contamination

1. Due to the sensitivity of nucleic acid amplification technologies precautions should be taken to avoid cross contamination of samples.
2. Avoid moistening the rim of the spin columns with pipette tips and avoid touching the column with the pipette tip.
3. Always use aerosol-barrier tips.
4. Avoid cross-contamination after each vortexing step, briefly centrifuge the tubes to remove droplets that may be on the lids of the tubes.
5. Close the lids of the spin columns before placing in the microcentifuge.
6. Flow-through generated after each centrifugation step may contain hazardous materials and should be disposed of appropriately.
7. Only open one spin column at a time and avoid creating aerosols.
8. Discard used processing tubes containing flow-through and place the new processing tubes containing PAXgene spin columns directly in the microcentrifuge.

Avoiding Degradation of RNA

1. Do not use any plastic-ware or glassware without first eliminating RNase contamination.
2. Take care not to introduce RNase into the sample during or after the purification procedure.
3. It is optimal to use sterile RNase free disposable vessels and solutions while working with RNA. Microbiological aseptic technique is always optimal to use when working with RNA.
4. Wear latex or vinyl gloved while handling reagents, tubes, and samples to prevent RNase contamination from the skin or surface of the laboratory.
5. Change gloves frequently.
6. Keeps tubes closed whenever possible.
7. Keep purified RNA on ice.
8. Keep samples frozen below -80º C or lower for long term storage.

7.2 Extraction of RNA from blood samples – procedure

1. Treat all blood as potentially infectious.
2. RNA Extraction is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.
4. Follow the detailed procedure outlined in the PAXgene Blood RNA Kit Handbook for extraction of RNA. The instructions are provided with the product or can be obtained from http://www1.qiagen.com/literature/handbooks/PDF/DSP/PaxGene/1028434_Qiag_PAXgene.pdf
5. Immediately after the procedure, place extracted and resuspended RNA on ice.

7.3 Extraction of RNA from blood samples – sample storage

1. Place extracted RNA samples in storage boxes.
2. Place samples at -80º C or lower.
3. Avoid freeze thaw cycles.
4. Record the location of storage.

8.0 APPLICABLE REFERENCES, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; 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, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series.
4. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
5. 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
6. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp
7. SOP #: BIO-SOP-BLD-PRO-RNA. Blood Sample Processing November 20, 2006 Procure, Quebec Prostate Cancer Biobank
8. Qiagen PAXgene Blood RNA Kit Handbook

