


CTRNet Standard Operating Procedure Blood Derivatives: Extraction of RNA			
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Approved By:	CTRNet Management Group (CMG)	01-June-2012	
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1.0 PURPOSE

Genomic studies often utilize nucleic acids (DNA and RNA) derived from patient samples. When extracting and storing ribonucleic acid (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 biobanks to follow when extracting RNA from blood samples.

2.0 SCOPE

This 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 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 08.02.002 Blood Processing and Storage

3.7 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 that are responsible for extracting RNA from blood.

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

5.0 MATERIALS, 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	
RNA extraction Kit and instructions	
Biohazardous waste container and autoclave bags	
Storage Boxes	
RNase inhibitor to clean surfaces	

6.0 DEFINITIONS

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

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

7.1.1 Avoiding Cross Contamination

- a. Due to the sensitivity of nucleic acid amplification technologies, precautions should be taken to avoid cross contamination of samples.
- b. Many RNA extraction protocols use spin columns. Avoid moistening the rim of the spin columns with pipette tips and avoid touching the column with the pipette tip.
- c. Always use aerosol-barrier tips.
- d. Avoid cross-contamination after each vortexing step. Briefly centrifuge the tubes to remove droplets that may be on the lids of the tubes.
- e. Close the lids of the spin columns before placing in the microcentrifuge.
- f. Flow-through generated after each centrifugation step may contain hazardous materials and should be disposed of appropriately.
- g. Only open one spin column at a time and avoid creating aerosols.
- h. Discard used processing tubes containing flow-through into appropriate biohazardous waste containers

7.1.2 Avoiding Degradation of RNA

- a. Work on clean surfaces and when appropriate wipe down with an RNase inhibitor.
- b. Do not use any plastic-ware or glassware without first eliminating RNase contamination.

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- c. Take care not to introduce RNase into the sample during or after the purification procedure.
- d. 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.
- e. Wear latex or vinyl gloves while handling reagents, tubes, and samples to prevent RNase contamination from the skin or surface of the laboratory.
- f. Change gloves frequently.
- g. Keeps tubes closed whenever possible.
- h. Keep purified RNA on wet ice for processing and for aliquoting.
- i. Keep samples frozen below -80°C or lower for long-term storage.

7.2 Extraction of RNA from Blood Samples

- 7.2.1 Treat all blood as potentially infectious.
- 7.2.2 RNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the tumour biobank.
- 7.2.3 Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.
- 7.2.4 Document the method of RNA extraction. There are several commercially available RNA extraction kits available, follow the detailed procedure outlined in the appropriate commercial kit handbook.
- 7.2.5 Immediately after the procedure, place extracted and re-suspended RNA on ice.
- 7.2.6 It is preferable to aliquot RNA into several smaller aliquots to limit freeze thaw cycles.
- 7.2.7 A small aliquot should be removed prior to long-term storage for quantitation and/or quality control.
- 7.2.8 Place extracted RNA samples in storage boxes and record location
- 7.2.9 Place samples at -80°C or lower.

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>

9.0 APPENDICES

None

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
8.2.003	2008	JdSH	Revised to cover only extraction of RNA from blood cells
8.2.003	2011	TS	Section 5.0: Added Racks for PAXgene tubes, cryotubes and qiagen columns. Added Biohazardous waste container, autoclave bags. Added wet ice. Section 7.1: Step 8: Added comment regarding where to discard waste. Step 7: Added wet ice Section 7.2: Step 6 and 7: Added steps regarding aliquots for storage and quality assurance. Section 7.3: Step 3: Document number of freeze thaw cycles.
8.2.003 e1.1	June 2012	CMG	Grammatical and formatting throughout Definitions removed Revision History moved to bottom Reference links updates Updated SOP references Revised Section 5.0 Table Minor Revisions to Section 7.1 Section 7.2.4 deleted reference link Merged section 7.3 with 7.2 section.