Title	Nucleic Acid Extraction from Blood Specimens
SOP Code	SOP112_02
Effective Date	04-Jan-2016

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Mmm/yyyy

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline standardized procedures for biorepositories to follow when extracting nucleic acids from blood samples.

2.0 SCOPE

The SOP describes how RNA and DNA should be extracted from blood samples to avoid contamination, prevent degradation, and preserve molecular integrity. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

3.0 RESPONSIBILITIES

The policy applies to all biorepository personnel responsible for extracting RNA and DNA from blood.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

This procedure is intended to ensure that RNA and DNA are extracted from blood

samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity.

5.1 General extraction considerations

5.1.1. Due to the sensitivity of nucleic acid amplification technologies precautions should be taken to avoid cross contamination of samples.

5.1.2. Avoid moistening the rim of the spin columns with pipette tips and avoid touching the column with the pipette tip.

5.1.3. Always use aerosol-barrier tips.

5.1.4. Avoid cross-contamination after each vortexing step, briefly centrifuge the tubes to remove droplets that may be on the lids of the tubes.

5.1.5. Close the lids of the spin columns before placing in the microcentifuge.

5.1.6. Flow-through generated after each centrifugation step may contain hazardous materials and should be disposed of appropriately.

5.1.7. If hemolysis of the sample occurs, it may still be viable depending on downstream applications, and ensure hemolysis is documented.

5.1.8. Only open one spin column at a time and avoid creating aerosols.

5.1.9. Do not use any plastic-ware or glassware without first eliminating RNase or DNase contamination.

5.1.10. Take care not to introduce RNase or DNase into the sample during or after the purification procedure.

5.1.11. It is optimal to use sterile RNase-free or DNase-free disposable vessels and solutions while working with nucleic acids. Microbiological aseptic technique is always optimal to use when working with nucleic acids.

5.1.12. Wear latex or vinyl gloved while handling reagents, tubes, and samples to prevent RNase and DNase contamination from the skin or surface of the laboratory. Change gloves frequently.

5.1.13. Keeps tubes closed whenever possible.

5.1.14. Keep purified RNA on ice.

5.1.15. Keep samples frozen below -80º C or lower for long term storage.

5.2 RNA Extraction Procedure

5.2.1 Treat all blood as potentially infectious.

5.2.2. Have materials and equipment ready before starting the procedure. Have as many tubes and cryovials as needed labelled and ready.

5.2.3. Follow the detailed procedure outlined in the RNA extraction kit manual.

5.2.4. Immediately after the procedure, place extracted and resuspended RNA on ice.

5.2.5. RNA samples should be stored at -80º C or lower.

5.3 DNA Extraction Procedure

5.3.1. Treat all blood as potentially infectious.

5.3.2.1. Have materials and equipment ready before starting the procedure. Have as many tubes and cryovials as needed labelled and ready.

5.3.3. If the Buffy Coat has been previously frozen, thaw with gentle agitation in a 37ºC water bath.

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

5.3.5. Follow the detailed procedure outlined in the DNA extraction kit manual.

5.3.6. Genomic DNA can be stored for short term at 4ºC. For longer term storage keep DNA at -80º C.

6.0 REFERENCES

Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series.
http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf

Best Practices for Repositories Collection, Storage Retrieval and Distribution of Human Biological Materials for Research, 3rd Edition 2012, International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org

US National Biospecimen Network Blueprint
http://www.ndoc.org/about_ndc/reports/NBN_comment.asp

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

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP112_01	01-Aug-2012	Original version.
SOP112_02	04-Jan-2016	2.0 Scope: Clarification added. 5.1.7: Instructions added for hemolyzed samples. Updated references. Removed OTRN logo.