Title	Nucleic Acid Extraction from Blood Specimens
SOP Code	SOP112_01
Effective Date	01-Sep-2012

Site Approvals

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

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to follow when extracting nucleic acids from blood samples. The SOP does not describe detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

The SOP describes how RNA and DNA are extracted from blood samples. This procedure ensures 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.

3.0 RESPONSIBILITIES

This procedure applies to all biorepository personnel responsible for extracting RNA and DNA from blood.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

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 microcentrifuge.

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

5.1.7 Open only one spin column at a time, and avoid creating aerosols.

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

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

5.1.10 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.

5.1.11 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.

5.1.12 Keeps tubes closed whenever possible.

5.1.13 Keep purified RNA on ice.

5.1.14 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 Place extracted and resuspended RNA on ice, immediately after the procedure.

5.2.5 Store RNA samples at -80º C or lower.

5.3 DNA Extraction Procedure

5.3.1 Treat all blood as potentially infectious.

5.3.2 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 Store genomic DNA for the short term at 4ºC, longer term storage at -80º C.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, http://www.ctrnet.ca/operating-procedures

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP112_01	01-Sep-2012	Original version