Title	Assessing Quality of Nucleic Acids
SOP Code	SOP121_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 a minimum of testing and assessment that should be carried on tissue derivatives, such as nucleic acids (DNA and RNA) to maintain quality and standardization of material. This SOP does not describe detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

This SOP outlines minimum assessment and testing that should be in place to evaluate the quality of DNA and RNA extracted in the biorepository.

3.0 RESPONSIBILITIES

This procedure applies to all biorepository personnel who are responsible for assessing the quality of nucleic acids.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 Quality Assessment - General Considerations

5.1.1 Assessment of molecular integrity of the samples in the collection must be done on a percentage of the stored samples as deemed suitable.

5.1.2 Assessment of molecular integrity must be performed by a designated laboratory using established procedures developed for this purpose.

5.1.3 Develop and use a defined scoring system that allows for a ‘quality score' to be assigned to a tissue or molecular sample.

5.1.4 Use the score in the interpretation of the quality assessment results.

5.2 Quality Assessment - DNA by Spectrophotometric Measurements, Enzymatic Digestion, and Gel Electrophoresis

5.2.1 Assess DNA quality in 1% percentage of the blood and tumour tissue samples stored, as well as in 1% of the DNA extracted from the blood and tumour samples.

5.2.2 Take UV spectrophotometric measurements to determine the DNA concentration.

5.2.3 Develop a standard of measurement against which relative DNA degradation can be compared. Use a standard DNA such as commercially available human genomic DNA that has been aliquoted and appropriately stored (to prevent DNA degradation from freeze/thaw cycles).

5.2.4 The quality assessment includes testing the suitability of the test sample DNA for use in enzymatic reactions (such as Hind III digestion) and visual assessment following agarose gel electrophoresis. Use a control sample as a reference.

5.2.5 Assign a quality score to the tested sample based on DNA quantification analysis (OD 260/280 ratio, agarose gel electrophoresis and restriction digestion):

DNA quality based on UV spectrophotometry

OD 260/280 ratio	Assigned score
1.8-2	4
1.6-1.8	3
1.4-1.6	2
1.2-1.4	1
Less than 1.2	0

DNA quality based on electrophoresis of genomic DNA

Electrophoretic Integrity	Assigned score
Discrete high MW band	4
10kb smear	3
5kb smear	2
2kb smear	1
Less than 2kb smear	0

DNA quality based on enzymatic digestion of genomic DNA

Electrophoretic Integrity	Assigned score
Discrete digestion band pattern	2
No band pattern	0

5.2.6 Add up the score. A score of 10 is indicative of high quality DNA; a score below 7 is indicative of poor quality DNA.

5.3 Quality Assessment - DNA by PCR

5.3.1 The method consists of amplifying different length fragments of the B-Globin gene (a “housekeeping” gene). The maximum amplicon size positively correlates with DNA quality.

5.3.2 The test and review must be performed by an individual, qualified by experience and training to do so.

5.3.3 Use the following primers:

B-Globin: GH20
GAAGAGCCAAGGACAGGTAC B-Globin:
PC04 CAACTTCATCCACGTTCACC
B-Globin: RS42 GCTCACTCAGTGTGGCAAAG
B-Globin: KM29
GGTTGGCCAATCTACTCCCAGG B-Globin:
RS40 ATTTTCCCACCCTTAGGCTG
B-Globin: RS80 TGGTAGCTGGATTGTAGCTG

Primer pairs and expected amplicon lenghts: GH20 + PC04 = 268 base pairs (bp)
RS42 + KM29 = 536 bp RS40 + RS80 = 989 bp
KM29 + RS80 = 1327 bp

5.3.4 Use the following reagents for the PCR reaction master mix (adjust total volume to accommodate the total number of samples being tested):

Master Mix:
2.5 µL 10X Taq Buffer (such as Amersham #27-0799-05)
4.0 µL dNTP (1,25 mM of each, such as Amersham # 27-2035-01)
1.0 µL Primer pairs (diluted at 20pM each)
15.0 µL H_2O
0.5 µL Taq DNA polymerase 5X (such as Amersham #27-0799-05)
23.0 µL Total of the master mix + 2 µL of DNA (50-100 ng/µL) = 25µL per reaction

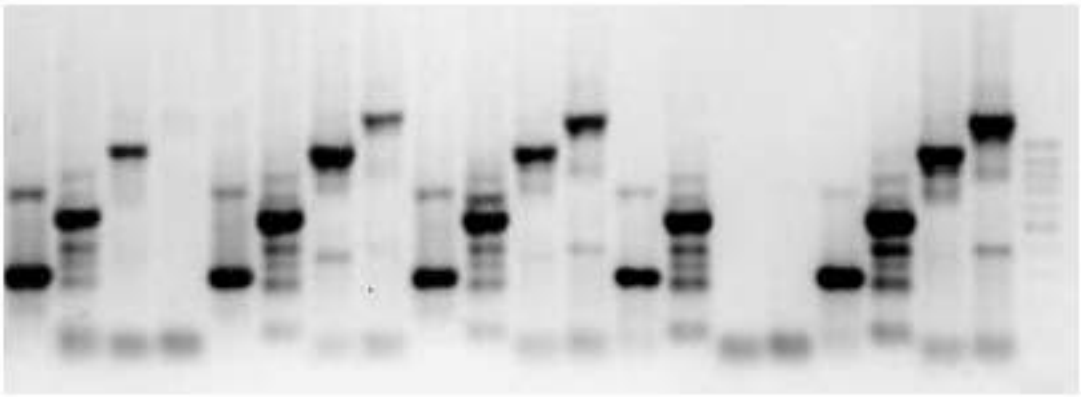
5.3.5 Use the following PCR reaction:

conditions: (3 min at 95) 1 cycle
(1 min at 95 , 2 min at 55 , 1 min at 72) 40 cycles
(5 min at 72) 1 cycle
(Optimized for PCR Thermo Hybaid MBS # HBMBSKIT2 adjust to suit alternate makes and model of thermocyclers)

5.3.6 Resolve on 1.2% agarose gel.

5.3.7 Sample results and scoring system for 4 primer pairs

Good	Very good	Very good	Poor	Very good
3 bands	4 bands	4 bands	2 bands	4 bands



5.4 Quality Assessment - RNA by Spectrophotometric Measurements and Gel Electrophoresis

5.4.1. Assess RNA quality in 1% percent of the blood and tumour tissue samples stored as well as in the RNA extracted from the blood and tumour sample.

5.4.2 Use a UV spectrophotometer measure the OD at 260 to determine RNA concentration.

5.4.3 Use the OD at 260/280 ratio to assess purity.

5.4.4 Run a small amount of the sample on a denaturing agarose gel to visualize the ribosomal RNA bands (28s and 18s). Crisp 28s and 18s bands are indicative of intact RNA. A 2:1 ratio of 28s:18s species has been considered the benchmark for intact RNA.

5.5 Quality Assessment - RNA by Using the Agilent Bioanalyzer

Note: The visual assessment described above is subjective and it is optimal to use an analytical tool such as the Agilent 2100 bioanalyzer (with RNA 6000 Nanoassay

Kit) to determine the concentration and purity/integrity of RNA samples. It provides a readout for sample quality and purity, has the added advantage of requiring small amounts of the sample and a quality score can be assigned based on the RNA integrity number value from the bioanalyzer.

5.5.1 Decontaminate Bioanalyzer Electrodes:

- Fill wells of the electrode cleaner with 350 µl of RNase ZAP and place in the bioanalyser for 1 minute.
- Remove and replace with another electrode cleaner filled with RNase-free water for 10 seconds.
- Remove and wait 10 seconds for the water on the electrodes to evaporate before closing the lid of the bioanalyzer.

5.5.2 Prepare the gel:

- Allow reagents to equilibrate to room temperature for 30 minutes before use.
- Place 550 µl of gel matrix into a spin filter and spin for 10 minutes at 1500 g.
- Aliquot 65 µl of the filtered gel into RNase-free microfuge tubes and store at 4° C until needed.

5.5.3 Prepare the gel-dye mix:

- Allow reagents to equilibrate to room temperature for 30 minutes before use.
- Vortex dye concentrate for 10 seconds and spin down to the bottom of the tube.
- Add 1 µl of the dye to a 65 µl aliquot of the filtered gel and vortex thoroughly.
- Spin for 10 minutes at room temperature at 13000g in a microfuge.

5.5.4 Load the gel-dye-mix:

- Place a new RNA nanochip on the chip priming station.
- Pipette 9 µl of the gel-dye mix at the bottom of the well marked G in black.
- Close the chip priming station and press the plunger until it is held by the syringe clip.
- Wait for exactly 30 seconds and release the plunger.
- Open the chip priming station and pipette 9 µl of the gel-dye into the other two wells marked G.

5.5.5 Load the marker:

- Pipette 5 µl of the RNA Nano Marker into the well marked with the ladder symbol and each of the 12 sample wells.

5.5.6 Load the ladder and samples:

- Pipette 1 µl of denatured ladder into the well marked with the ladder symbol.
- Pipette 1 µl of each of the denatured samples into each of the sample wells.

- Vortex the chip for 1 minute at 2400 rpm.
- Insert the chip in the bioanalyzer and start the instrument.

5.6 Quality Assessment - Records

5.6.1 Record test results for each quality assurance tested sample in the institution database or informatics system.

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
SOP121_01	01-Sep-2012	Original version