National
Liver
Disease
Biobank

Standard Operating Procedure

DOCUMENT NAME: QUALITY ASSURANCE PROCEDURES

DOCUMENT NO. : ILBS#NLDB:E

Document Name	**:**	**Assessing Quality of Tissue Specimens**
Document No.	**:**	**ILBS#NLDB:E.1**
Version No.	**:**	**1.0**
Effective Date	**:**	**01/01/2025**

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Number	Effective date	Pages	Author	Authorized by
SOP: NLDB:E.1	01/01/2025	5	Mr. Satish Kumar	Dr. Chhagan Bihari
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1.0	2yrs	3	Dr. Chhagan Bihari	30/12/2024

Location	Subject
Biobank Reception area Almira	Assessing Quality of Tissue Specimens.
Function	**Distribution**
To give information about: testing procedures should be in place to monitor and assess the quality of the samples in the collection.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP outlines the minimum assessment required to evaluate the quality of samples stored in the biobank.

RESPONSIBILITY:

This SOP applies to NLDB personnel involved in tissue quality assessment, where the Pathologist performs histopathological characterization and the Technician conducts quality assurance procedures and maintains documentation

1.0 PURPOSE

Quality assurance is fundamental to the successful operation of a biobank offering biospecimens for research purposes. A high standard of sample quality is essential to avoid introducing inconsistencies and variables into research studies. NLDB should be confident that they are providing samples with the appropriate quality to meet the research needs of investigators. Testing procedures should be in place to monitor and assess the quality of the samples.

2.0 SCOPE

This SOP outlines the minimum assessment required to evaluate the quality of samples stored in the biobank in order to provide investigators with a product that is consistent with their needs. This SOP does not cover an assessment of molecular quality.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB that are responsible for assessing the quality of tissue specimens.

Pathologist: Conduct histopathological characterization.

Technician: Conduct and assist with quality assurance procedures; Records and documents outcomes.

4.0 MATERIALS, REAGENTS EQUIPMENT AND FORMS

- Markers, ink and pens
- Eosin
- Haematoxylin (filtered)
- Microscope
- Slides

5.0 PROCEDURES

The research and scientific utility of the data obtained from the analysis of sample depends on the quality of the sample. These procedures outline minimum steps that should be followed to ensure that the samples collected, stored, and distributed are of sufficient morphological calibre to meet the research needs of the investigators.

5.1 General considerations for morphological review

The biobank should ensure that morphological review to determine sample composition is conducted by the biobank or that the researcher knows that it has not been conducted.

5.1.1 At a minimum, assessment must consist of morphologic review of all collected tissue [frozen and formalin fixed paraffin embedded (FFPE)] samples (including archival material).

5.1.2 Use researcher feedback about sample quality to refine collection and storage practices and guide evolution of quality control procedures.

5.1.3 Use a defined scoring system that allows researchers to interpret whether the tissue is suitable for the proposed assay.

An empirical example of a scoring system for frozen tissue is as follows:

5.1.4 A gold level specimen will be harvested directly from the participant, from either an intra-operative biopsy or as soon as possible (<15 min) following surgical resection.

5.1.5 A silver level specimen will be harvested as soon as possible following specimen removal within a range of 15 to 30 minutes post resection.

5.1.6 A bronze level specimen will be harvested within two hours of surgery (30 min to 120 min), either in the operating room or in the pathology specimen cutting room.

5.1.7 A test level specimen is one where the time from surgical resection to harvesting is unknown or longer than 2 hours.

5.2 Quality assessment – Pathology review

5.2.1 Basic quality control practice must include a morphologic review of Formalin fixed, Haematoxylin and Eosin **(**H&E) stained slide, representative (mirror-image, adjacent) of the snap frozen or formalin fixed paraffin embedded tissue sample (for each relevant FFPE block).

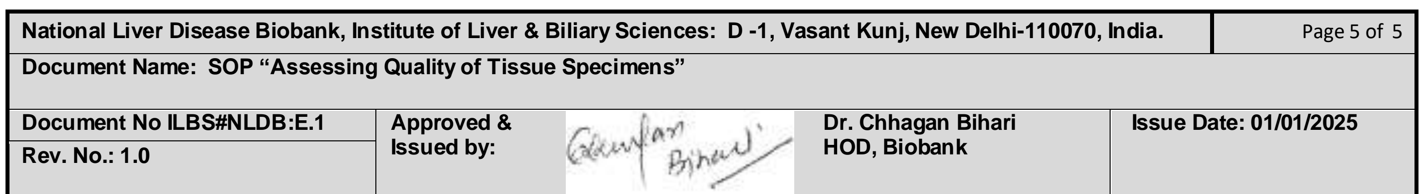
5.2.2 The review must be performed by a qualified individual.

5.2.3 It is suggested that the review confirm and assess:

- Tissue type and assessment of diagnosis
- Tumour type& grade
- Presence of tumour
- Percent cellularity of tumour and stroma
- Percent necrosis or signs of degradation
- Presence of inflammatory cells

5.2.4 Ideally if digital imaging is available, a digital image of an area representative of the tissue sample should be stored in the biobank database.

5.2.5 Record Results of review in database.



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DOCUMENT NAME: QUALITY ASSURANCE PROCEDURES

DOCUMENT NO. : ILBS#NLDB:E

Document Name	**:**	**Assessing Quality of Nucleic Acids**
Document No.	**:**	**ILBS#NLDB: E.2**
Version No.	**:**	**1.0**
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Location	Subject
Biobank Reception area Almira	Assessing Quality of Nucleic Acids
Function	**Distribution**
To assess the quality and integrity of extracted DNA and RNA samples to ensure suitability for downstream research and storage applications.	➢ HOD ➢ Biobank Reception area ➢ Master files

SCOPE AND APPLICATION:

This SOP defines the minimum quality assessment requirements for DNA and RNA extracted in the biobank to ensure suitability for intended research use.

RESPONSIBILITY:

The SOP applies to all personnel from NLDB member biobanks who are responsible for assessing the quality of nucleic acids.

1.0 PURPOSE

Quality assurance is fundamental to the successful operation of a biobank offering tissue specimens and derivatives for research purposes. A high level of molecular integrity is essential for avoiding inconsistencies and variables in research studies. Nucleic acid quality is critically important for many techniques utilized in genomic analysis, for the meaningful interpretation of results and for the facilitation in the comparison of results across independent laboratories. Ideally, testing procedures should be in place to monitor and assess the quality of the samples in the collection.

2.0 SCOPE

This SOP outlines minimum assessment and testing that should be in place to evaluate the quality of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) extracted in the biobank in order to provide investigators with a product that is consistent with their needs.

3.0 ROLES AND RESPONSIBILITIES

The SOP applies to all personnel from NLDB member biobanks who are responsible for assessing the quality of nucleic acids.

Technician: Conducts and assists with quality assurance procedures; Records and documents outcomes.

4.0 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

- UV Spectrophotometer and quartz cuvettes
- Reagents for PCR reaction and Bioanalyzer
- Thermocycler for PCR reaction
- Agilent Bioanalyzer 2100
- Appropriate tubes
- RNA 6000 Nano Kit

5.0 PROCEDURES

The research and scientific utility of the data obtained from the analysis of nucleic acids correlates specifically with the molecular integrity of the extracted DNA or RNA. Degraded or contaminated nucleic acid samples will lead to inconsistent or unreliable results. Confounding factors (termed pre-analytic variables) influence the quality of the extracted nucleic acids. These include, for example, physiological state of the tissue prior to harvesting, post-resection interval from collection to preservation and storage conditions.

The following procedures provide an example of steps that may be used by a biobank to assess the molecular calibre of the samples in the collection:

5.1 Quality assessment – General considerations for molecular assessment of nucleic acids

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 Use researcher's feedback about sample quality to refine collection and storage practices and guide evolution of quality control procedures.

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 that has undergone assessment at a designated quality control laboratory.

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

5.2 Participant file creation and maintenance

5.2.1 Extract/isolate DNA and document protocol used.

5.2.2 Take UV spectrophotometric measurements to determine the DNA concentration and OD 260/280 ratio.

5.3 Quality assessment – DNA by Polymerase Chain Reaction (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 lengths:

- 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:

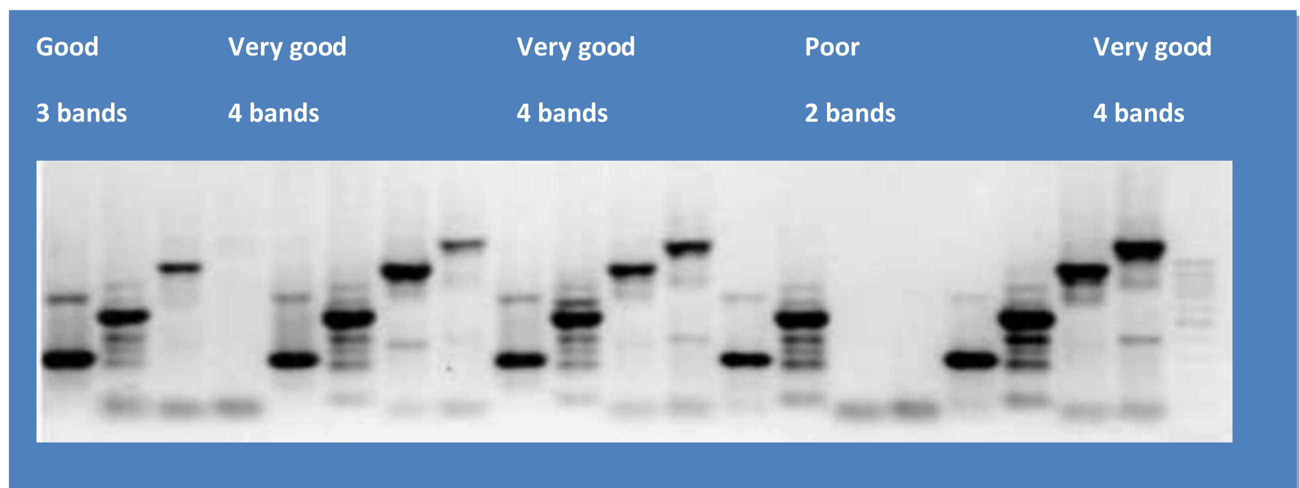
- 2.5 µL 10X Taq Buffer (such as Amersham #27-0799-05)
- µLdNTP (1, 25 mM of each, such as Amersham # 27-2035-01)
- µL Primer pairs (diluted at 20pM each)
- 15.0 µL H2O
- 0.5 µLTaq DNA polymerase 5X (such as Amersham #27-0799-05)
- 23.0 µL Total of the master mix + 2 µL of DNA (50-100 ng/µL) = 25µL per 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.



5.4 Quality Assessment – RNA by spectrophotometric measurements

5.4.1 Extract/isolate RNA and document protocol used.

5.4.2 Take UV spectrophotometric measurements to determine the RNA concentration and OD 260/280 ratio.

5.5 Quality Assessment – RNA by using the Agilent Bioanalyzer

The following procedure is used by the quality control centre of NLDB and is based on the use of the Agilent 2100 Bioanalyzer (with RNA 6000 Nano assay Kit or suitable commercial kits) to determine the concentration and purity/integrity of RNA samples. It provides a read-out 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**

a. Fill wells of the electrode cleaner with 350 µl of RNase ZAP and place in the Bioanalyzer for 1 minute.
b. Remove and replace with another electrode cleaner filled with RNase-free water for 10 seconds.
c. 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**

a. Allow reagents to equilibrate to room temperature for 30 minutes before use.
b. Place 550 µl of gel matrix into a spin filter and spin for 10 minutes at 1500 g.
c. 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**

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

5.5.4 **Load the gel-dye-mix**

a. Place a new RNA Nano chip on the chip priming station.
b. Pipette 9 µl of the gel-dye mix at the bottom of the well-marked G in black.
c. Close the chip priming station and press the plunger until it is held by the syringe clip.
d. Wait for exactly 30 seconds and release the plunger.
e. 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.**

a. 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 **Loading the ladder and samples**

a. Pipette 1µl of denatured ladder into the well-marked with the ladder symbol.
b. Pipette 1 µl of each of the denatured samples into each of the sample wells.
c. Vortex the chip for 1 minute at 2400 rpm.
d. 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.

5.6.2 Include in the test results the SOP used.

Below are diagrams displaying High Quality RNA, Marginally degraded RNA, and Highly degraded RNA.

A. Electropherogram showing High Quality RNA (RIN 9)

B. High quality RNA is characterized by clear 28S and 18S peaks, low noise between the peaks and minimal low molecular weight contamination.

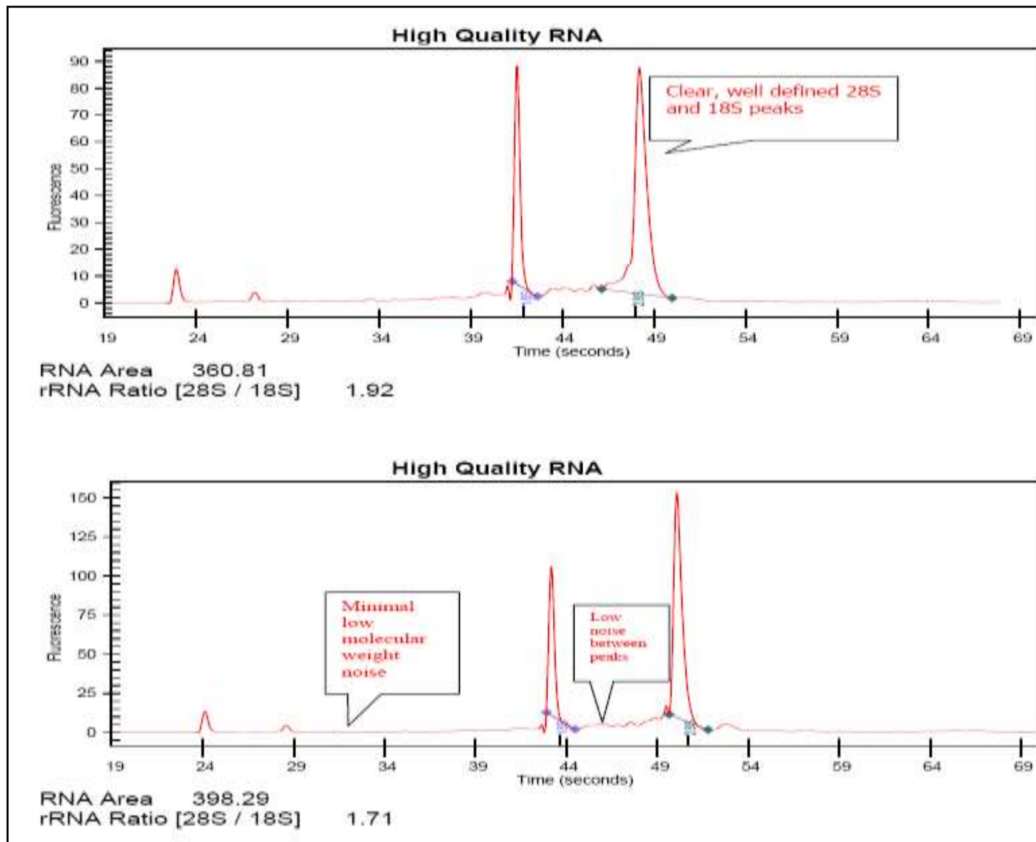


Figure 1: