SBP Recommendation

Preanalytical conditions to prepare human RNA from whole blood

Introduction

Pre-analytical factors include all factors that can influence the results of a sample analysis, particularly during the processes of sample collection, transport, processing, and storage. Their impact, often underestimated, can be critical. Identifying and controlling these factors at each step, and standardizing processes to obtain reliable, reproducible, and accurate results, will help ensure sample quality and suitability for advanced research applications, particularly for omics studies. Indeed, the integration of multi-omics data, facilitated by technological advancements, is crucial for accelerating both research progress and advancements in precision medicine. In this context, ensuring the reliability and integrity of biological samples is essential and biobanks and researchers play a key role in this.

The aim of this document is to support and guide researchers and biobanks to prepare high-quality RNA for transcriptomic projects.

Before considering each process in detail and focusing on sample quality, you need to establish standardized procedures for tracking samples effectively (incl. naming, coding) and mitigate the risk of sample swapping.

For each pre-analytical process, from collection to storage, a set of recommendations including RNA quality criteria is given, highlighting which steps are important when handling samples to preserve the integrity of the RNA, minimize the possibility of any chemical modifications, and avoid any agents that could contaminate nucleic acid samples or inhibit downstream enzymatic manipulations (e.g., residual proteins, chemical reagents). These recommendations are aligned with the ISO 20186-1: Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 1: Isolated cellular RNA[1].

Compared to DNA, RNA is particularly unstable and dramatic changes in RNA profile can occur within minutes of blood collection. Precautions must be taken even more diligently when handling and storing both starting material and pure RNA.

To further support researchers and biobanks, a list of key pre-analytical variables to be documented is provided at the end of the document.

FACTORS INFLUENCING RNA QUALITY & RECOMMENDATIONS [i]

SAMPLE COLLECTION

Collection tube is a critical factor in the blood collection process. The **type of tube material** as well as the **additives** used to preserve the blood are factors to be considered to ensure RNA preservation.

We recommend:

1) Use a **commercially available collection tube** containing a blood RNA profile stabilizer (e.g. PAXgene Blood RNA tube, Tempus Blood RNA tube). The use of blood collection tubes without stabilizer is strongly discouraged.
2) **Do not underfill or overfill the tube** to maintain the correct ratio of additives. Care must be taken when filling the tube to respect the recommended volume.
3) Homogenize blood with additives. To avoid RNA degradation, **homogenization must be carried out gently. Do not shake or spin the tube.** The mixing of blood and additives is often achieved by inverting the tube repeatedly.
4) Always respect and follow the instructions provided by manufacturer **carefully and precisely**.

SAMPLE TRANSPORT / RECEPTION

Time and temperature are major factors of variability of these two pre-analytical steps.

We recommend:

1) Document the storage duration of biological material prior to processing. If collection and processing do not take place on the same site, the overall storage duration includes, if applicable, the duration of storage at the collection site, the duration of transport and the duration of storage prior to extraction at the processing site.
2) Follow the manufacturer's instructions regarding the maximum duration before treatment permitted. In all cases, **maximum storage duration must be controlled and kept as short as possible.**
3) Respect and document temperature conditions. For immediate extraction and up to 72 hours, **keep the blood sample at 4°C**[2] and maintain the cold chain until processing. If the extraction cannot be immediate, process the blood sample as quickly as possible.
4) **Do not freeze the blood sample**, as this considerably damages the RNA[3].

SAMPLE PROCESSING

During processing, RNA is extracted from the blood sample. Precautions must be taken to avoid any contamination that could affect the quality of the RNA. The method used is also key in this process.

We recommend:

1) Carry out nucleic acid isolation according to a precise, validated and verified protocol. In this case, refer to SBP SOP on method validation (available here). We however strongly recommend using **commercially available isolation kits** and follow the manufacturers' instructions.
    a. If the manufacturer of the blood collection tube specifies a kit to be used, follow its recommendations.
    b. If RNA isolation is carried out by an automated method, using a robot, follow the manufacturer's

[i] In all cases, and where applicable, the manufacturer's instructions must be followed precisely.

instructions concerning the specific kits to be used.

c. If a manufacturer's protocol needs to be adapted, validation is required.

2) Perform RNA isolation in a **separate area** from the amplification step, to avoid cross-contamination.

3) Strictly use **RNase-free surfaces, reagents, and consumables during processing** to avoid RNase contamination and thus RNA degradation, as RNA is highly sensitive to degradation by RNase.

4) Include **a DNase step** to remove any remaining DNA.

5) Elute nucleic acid in an **appropriate buffer included in the kit or recommended** by the manufacturer.

6) For storage of pure RNA, use **specific cryogenic tubes** to reduce nucleic acid absorption.

RNA STORAGE

The duration and temperature of final storage are factors to be taken into account to ensure long-term RNA quality, if it is not immediately analyzed.

We recommend:

1) Follow the kit manufacturer's instructions on storage conditions.

2) If there are no recommendations:

   a. For immediate assay, keep extracted RNA at 4°C.

   b. For short- and long-term storage, RNA should be stored in ultra-low freezer **(range between -70°C and -80°C).** If possible, it is recommended to **generate aliquots** to avoid repeated freeze/thaw cycles, which could lead to RNA degradation.

3) Implement temperature monitoring and automated alarming system to check storage temperature, to ensure the safety of frozen samples.

RNA SAMPLE REQUIREMENTS

Prior to nucleic acid analysis, it is essential to assess its quality which can directly impact the success of the analysis.

Determining RNA quality involves measuring its concentration, purity (indicator of contamination) and integrity (state of RNA degradation).

We recommend:

1) **Concentration**: Quantify RNA by a sensitive and precise analysis using specialized methods such as fluorescent dye quantification[4]. Alternatively, measurement by spectrophotometry is also possible. It should be noted that differences in concentration may be observed for the same sample, depending on the method used. Recommended values depend on subsequent analyses.

2) **Purity:** Measure by spectrophotometry

   a. the A260/280 ratio, an indicator of contamination by proteins or DNA. A ratio of ~2.0[4,6] is generally accepted as 'pure' for RNA, and a range between 1.9 and 2.1 (in 10 mM Tris·Cl pH 7,5) is accepted.

   b. the A260/230 ratio, an indicator of contaminants that absorb at 230 nm such as proteins, guanidine HCL, EDTA, carbohydrates, lipids, salts, or phenol[4]. A ratio between 2.0 and 2.2[6] is accepted.

   Note that the 260/280 ratio may vary according to pH[5]. We recommend using the same solvent so that absorbance readings are consistent from one measurement to the next.

3) **Integrity**: Measure the RNA Integrity Number (RIN), which is an algorithm for assigning integrity values to RNA measurements[4] obtained using electrophoresis-based method. RIN values are distributed on a scale of 1 to 10. A high RIN indicates highly intact RNA, and a low RIN indicates highly degraded RNA sample. A value above 7[4,7] indicates good quality RNA, but recommended values may depend on subsequent analysis.

PREANALYTICAL VARIABLES TO BE DOCUMENTED - RNA SAMPLES

Below is the list of the key variables to be documented as part of the standardization of data documentation to accompany your RNA sample and record key preanalytical data. For a complete list of variables, please refer to the document "SBP Dataset for Human Liquid Sample"[8].

Process	Variables	Description
Collection	`sample_ID`	Unique identifier of primary sample
	`collection_start_time`	Date and time of sampling
	`sample_type`	Type of sample
	`primary_container_type`	Type of primary container in which the sample is collected
	`container_volume`	Volume of primary container in which sample is collected
	`collection_special_conditions`	Additives or specific conditions applied
	`pre_transport_temp`	Temperature conditions before transport
Transport	`transport_start_time`	Date & time when transport starts
	`transport_temp`	Temperature conditions during transport
Reception	`reception_time`	Date & time when sample arrives at reception
Processing	`processing_start_time`	Date & time when processing starts
	`sample_additive`	Type of sample additive
	`isolation kit`	Isolation kit for RNA isolation
	`storage_container`	Storage container type for long term storage
	`freezing_mode`	Freezing mode of the sample
	`freezing_start_time`	Date and time of freezing
Quality measurements	`quantification_method`	Type of quantification method used
	`concentration`	Concentration value obtained (standard unit: ng/µL)
	`RIN`	RIN value obtained
	`A260/A280 ratio`	A260/A280 ratio value obtained
	`A260/A230 ratio`	A260/A230 ratio value obtained
Storage	`storage_start_time`	Date & time when sample is physically stored in the freezer – can be the same as freezing time
	`storage_temperature`	Storage temperature
	`storage_place`	Storage location of the sample
	`freeze_thaw_cycle`	Actual sample status - e.g. "2" = defrosted twice

References

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8. SBP datasets for human liquid samples – Version 2.0

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