RNA EXTRACTION

Responsable(s):

- **Ângela Afonso** – Sala P0-C-77; Ext. 47047/92903; email: angelaafonso@fm.ul.pt
- **João Eurico Fonseca** – Sala P2-A-18; Ext. 47221; email: jcfonseca@fm.ul.pt

AUTHOR: Ângela Afonso

APPROVAL: João Eurico Fonseca

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1. SCOPE

This Standard Operating Procedure defines the isolation and storage protocols of total RNA from blood. The blood destined for Biobanco-IMM will be collected only from patients who have freely given their informed consent.

2. SAFETY INFORMATION

All specimens should be treated as infectious and handled according to "standard precautions".
Specimens must be processed only by trained staff. White coat, gloves, safety glasses and other individual protection devices must always be worn while collecting and handling samples.

A. Interferences

Immediate stabilization of RNA is a critical step due to the changes in the gene expression pattern which occur immediately after harvest. Such changes must be avoided for reliable analyses, such as biochip, arrays and quantitative RT-PCR.
Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Because RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA use only RNases-free material, sterile filter-tips for pipettes and adopt aseptic techniques.

4. DESCRIPTION / PROCEDURE

A. Specimen identification

The patient's specimen must be unambiguously identified at the time of collection. Specimens should be labeled and handled in a manner that respects patient privacy according the law n.º 12/2005, published at *Diário da República.*
Each tube must be labeled with an identifier that links with the donor's unique identification number; this ensures traceability of the specimen and separation of personal and clinic data.

B. Blood collection and stabilization of intracellular RNA

Blood samples should be collected in Paxgene tubes for RNA stabilization. For use and storage follow the instructions of the manufacturer (in case of storage it is recommended to split stored biospecimens into two sets of aliquots, each set stored in a different location; this strategy will avoid loss in case of adverse events in one location).

C. Transport to the laboratory

- Transport at 18-25 ºC. The sample must arrive to the laboratory within 3 days from the blood collection;
- Transport at 2-8 ºC. The sample must arrive to the laboratory within 5 days from the blood collection;
- Storage at -40ºC to –80 ºC (the sample is stable for 4 years).

For the transport of the fresh blood samples to laboratory, see SOP002.

D. Processing procedure

i) Check that all specimens and relative documentation are present; If something is missing or the documentation or accompanying labels are incomplete, illegible or mismatched, contact the collection center. In this case, the Biobanco-IMM will put the samples on "stand-by". If the missing information is not sent to the Biobanco-IMM we reserve the right to reject and discard the specimens. In this case it will be necessary to record the "not conformity" in the Biobanco-IMM database.

ii) Register the tubes in the Biobanco-IMM database; data of each specimen must be recorded in electronic and paper archive.
iii) The Paxgene tubes can be processed or stored at –80ºC (for a maximum of 4 years). Record storage details in the Biobanco-IMM database.
iv) *RNA isolation*: RNA isolation can be performed by manual procedure or by using an automated procedure employing robotic workstations. RNA purification is performed according to the manufacture's protocol.
Researchers who send to the Biobanco-IMM purified RNA, must declare the extraction procedure, the date of the extraction, the storage temperature of these samples and the concentration of the RNA.
v) *RNA quantity and quality controls*: RNA concentration should be determined after the extraction protocol in the eluted solution, measured by absorbance at 260 nm. To ensure significance, readings should be in the linear range of the spectrophotometer. The ratio of the readings at 260 nm and 280 nm (A260/A280) provides an estimate of the *purity of RNA* with respect to contaminants that absorb in the UV, such as proteins. Since the A260/A280 ratio is influenced considerably by pH, for accurate values, measure the absorbance in 10 mM Tris-HCl, pH 7.5. Pure RNA has an A260/A280 ratio of 1.8-2.2 in 10 mMTrisCl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution. The 260/230 ratio is a second measure for purity of the sample, as the contaminants absorb at 230nm (like EDTA). The A260/A230 ratio should be higher than the A260/A280 ratio, as it is usually between 2 and 2.2. Lower ratio might be an indication of contamination.
vi) *The integrity and size distribution:* of total RNA can be checked by denaturating agarose gel electrophoresis and ethidium bromide staining or by using a bioanalyzer. The respective ribosomal bands should appear as sharp bands or peaks on the stained gel. 28S ribosomal RNA bands should be present with intensity approximately twice that of the 18S RNA band. If the ribosomal bands or peaks in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample have suffered major degradation during preparation.
vii) The processing details must be recorded in the Biobanco-IMM database.

E. Long term storage of RNA samples

After the RNA purification and quantity and quality controls, purified RNA must be aliquoted in a 1.2 ul tubes and preserved in –80 ºC freezer. Under this condition no degradation of RNA is detectable after 1 year. The storage details must be recorded in the Biobanco-IMM database.

F. Backup

It is recommended to split biospecimens into two sets of aliquots, each set stored in a different location; this strategy will avoid loss in case of adverse events in one location.

G. Transport of frozen samples

Frozen RNA samples must be transported at -80 ºC; it's critical to maintain the cooling conditions at all times during transport and storage.
The tubes must be transported upright and secured in a leak-proof secondary receptacle. Cushion or suspend tubes during transport. There should be sufficient adsorbent paper around tubes to soak up all liquid in case of a spill. Finally, there should be an outer packaging of adequate strength for its capacity. Dry ice should be placed around the secondary packaging or alternatively in an over pack with one or more complete packages.
In the external container the following information should be legible: the name, phone number and address of the Biobanco-IMM; the contact person ; the receiver; the label "human biological material", "handle with care"; the biohazard symbol and the presence of cryogenic gas.
Special requirements should be provided to couriers responsible for the transport of the specimens.
Enclose a form containing: number and type of samples with their unique identification codes, diagnosis of donor; date and details of production; date of shipped; temperature of transport; expiry

date; notes; instructions for the opening of the packaging and the sample container and information about the presence of cryogenic gas.
The receiving laboratory must be informed about the shipment and probable date of arrival of the samples. Once arrived, the receiving laboratory should communicate to the Biobanco-IMM the arrival of the samples.
The transport details must be recorded in the Biobanco-IMM database

H. Stability of the purified RNA

The purified RNA is stable for 1 year at –80ºC.

5. RECORDS

Records' Identification	Indexation	Archive Responsible
FORM.BIO.001	Base de dados LIMS	Ângela Afonso
Questionares	Base de dados LIMS	Ângela Afonso

6. INFORMATION

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B. Documentation:

- **SOP** – SOP.BIO.002