General remarks

RNAses are ubiquitous, unusually stable and potent enzymes. Hence, only clean and sterile collection tubes should be used. Since RNAses are present in the collected tissue, they have to be inhibited as soon as possible after tissue collection. In fact, every second counts to preserve the valuable RNA.

Various methods (see below) for the preservation of biopsy specimen for later RNA preparation have been employed successfully.

Methods

A. Storage in liquid nitrogen (lN_2)

Immediately after collection from the patient, the tissue sample should be transferred to a screw-cap tube and snap-frozen in lN_2. (The tube should contain a small hole on top for evaporation of lN_2. Otherwise, the tube could explode upon removal from the lN_2 container. For safety reasons, one should wear safety goggles and/or a face protection shield).

In lN_2, storage for years is possible. Alternatively, the snap-frozen sample can be stored in a freezer at –80°C . It must not thaw, however, even not for seconds.

B. Storage in RNAlater (Qiagen)

RNAlater (Qiagen, cat. no. 76104 or 76106) is a liquid preservative for tissue samples.

Immediately after collection from the patient, the tissue sample should be transferred to an Eppendorf tube containing at least 10 volumes (relative to the volume of the biopsy specimen) RNAlater. The tube content should immediately be mixed thoroughly and the sample kept submerged in the preservative. According to Qiagen, storage at –20°C for 3 months (or 4°C for 4 weeks) without loss of RNA is possible. For RNA preparation, the tube should shortly be centrifuged and the RNAlater supernatant thoroughly be removed by pipetting.

C. Blood collection and storage with the PAXgene Blood RNA Tube (Qiagen; cat. no. 762125) for subsequent RNA preparation

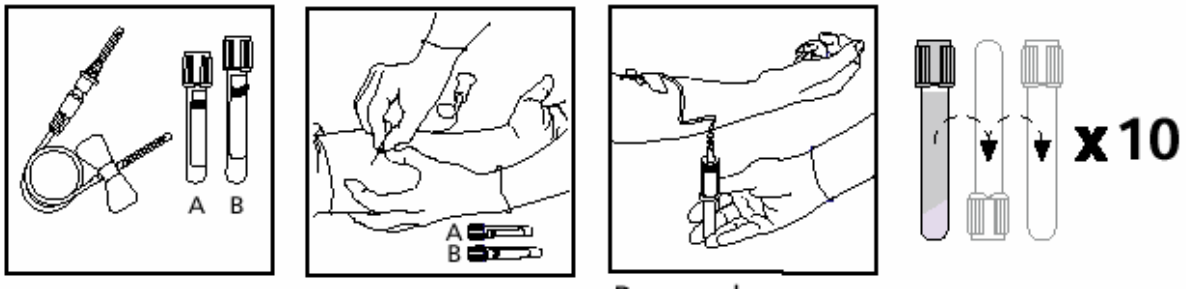
Required Blood Collection Accessories (Not included with PAXgene System)

1. A BD Safety-Lok™ blood collection set
2. Any BD Vacutainer™ needle holder of the standard size may be used with 16 mm diameter tubes.
3. A "Discard Tube" if the PAXgene Blood RNA Tube is the only tube being drawn.
4. Alcohol swab for cleansing site.
5. Dry sterile gauze.
6. Tourniquet.

7. Needle disposal container for used needle or needle/holder combination.

Procedure

1. Ensure that the PAXgene Blood RNA Tube is at room temperature (18-25°C) prior to use and properly labeled with patient identification.
2. If the PAXgene Blood RNA Tube is the only tube to be drawn, draw into a "Discard Tube" prior to using the PAXgene Blood RNA Tube. Otherwise, the PAXgene Blood RNA Tube should be the last tube drawn.
3. Using a blood collection set, collect blood into the PAXgene Blood RNA Tube using your institution's recommended procedure for standard venipuncture technique.
4. Hold the PAXgene Blood RNA Tube vertically, below the blood donor's arm during blood collection.
5. Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.
6. After blood collection, gently invert the PAXgene Blood RNA Tube 8 – 10 times.
7. Store the PAXgene Blood RNA Tube upright at room temperature (18-25ºC) or 4ºC (possible for up to 5 days).



A- Discard Tube; B- PAXgene Blood RNA Tube

Draw order: Tube A, Tube B

Version	Tracking of changes	Name	Date