Evaluation of the PAXgene™ Bone Marrow RNA System for stabilization and purification of cellular RNA

PreAnalytiX
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1. Introduction

Gene expression analysis of human bone marrow samples is becoming an important tool in clinical research, diagnostics, and disease monitoring (e.g., minimal residual disease, leukemia). Ex vivo changes in gene expression, starting at the time of bone marrow aspiration, pose a major challenge for reliable analysis. PreAnalytiX has recently developed and launched a "For Research Use Only" (RUO) system that enables the collection, stabilization, storage, and transportation of human bone marrow specimens, together with a rapid and efficient protocol for isolation and purification of cellular RNA. The system comprises bone marrow tubes (PAXgene Bone Marrow RNA Tube [50], cat. no. 764114) and an RNA preparation kit (PAXgene Bone Marrow RNA Kit [30], cat. no. 764133).

The aim of this study was to evaluate and verify the basic performance characteristics of the new PAXgene Bone Marrow RNA System (RUO) as compared with unstabilized bone marrow and a reference RNA isolation method. In detail, prepared RNA samples were analyzed for RNA yield, purity, integrity, relative levels of selected transcripts, and residual genomic DNA to specify basic product performance.

Materials and methods

In accordance with individual institutional procedures, a total of 295 bone marrow samples were aspirated from patients of unselected age with different diagnoses and treatments at several medical centers and clinics. Aspiration was done following in-house standard procedures using commonly used aspiration devices, syringes, and different anticoagulants (e.g., EDTA, heparin, citrate). Sample aliquots (2 ml) were transferred into PAXgene Bone Marrow RNA Tubes stored and transported at different temperatures (up to 3 days at 18–25°C, 5 days at 2–8°C, several weeks at –20°C or –70°C), and processed using the PAXgene Bone Marrow RNA Kit according to manufacturer's handbook.

2. Materials and methods (continued)

Prepared RNA was analyzed for yield, purity, integrity and residual genomic DNA (gDNA) using UV spectroscopy, capillary gel electrophoresis with calculation of RNA integrity numbers (RIN, Agilent® 2100 Bioanalyzer with Nanochips), and real-time monoplex PCR assay of ,-actin sequences (QIAGEN® QuantiTect® PCR system) to quantify traces of genomic DNA with standard curve method on an SDS 7700 instrument (Applied Biosystems). Of the total 295 samples, only 265 samples were analyzed for RNA purity.

For a specimen subset of larger aspiration volumes available, anticoagulated quadruplicate parallel bone marrow sample aliquots were transferred into two PAXgene Bone Marrow RNA Tubes or left untreated (two reference aliquots). From each pair of aliquots, the first one was processed directly using the PAXgene Bone Marrow RNA Kit or the reference method, a standard acid phenol organic extraction (QIAzol® Lysis Reagent) with silica-membrane–based RNA cleanup (QIAamp® RNA Blood Mini Kit). Remaining corresponding second aliquots were stored at room temperature for 2 days prior to processing as described.

In addition to the RNA analysis described above, those samples were used to investigate applicability of RNA to downstream analysis using RT-PCR. Transcripts of IL-8, p53, cox-2, IL-1β, and c-fos were selected as marker transcripts to monitor transcript profiles over time of storage and to verify the stabilization properties of the PAXgene Bone Marrow RNA System in comparison with the reference method. One-step real-time monoplex RT-PCR assays were optimized and used to compare transcript levels between paired RNA samples from tubes without and with storage prior to RNA preparation.

3. Results — Transcript stability in bone marrow samples at room temperature

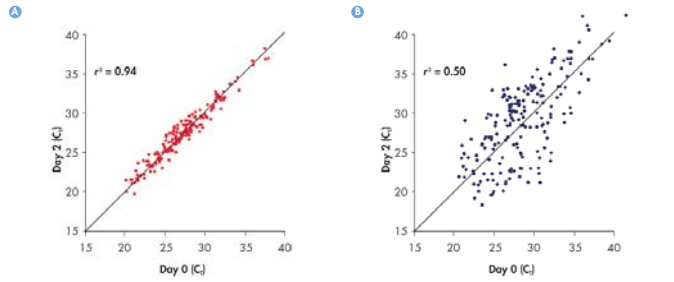

Figure 1 Bone marrow samples were collected from 39 individuals and immediately transferred to A PAXgene Bone Marrow RNA Tubes or B EDTA tubes. RNA was purified using the PAXgene Bone Marrow RNA Kit or reference method, a standard acid phenol organic extraction with silica-membrane–based RNA cleanup at day 0 or after 2 days storage at room temperature. Expression levels of 5 marker transcripts for each sample (IL-8, p53, cox-2, IL-1β, c-fos) were quantified using real-time RT-PCR. The high correlation of C_T values (threshold cycles) for the samples stabilized in PAXgene Bone Marrow RNA Tubes indicates that the levels of these transcripts did not significantly change during storage, in contrast to the samples stored in EDTA tubes.

4. Results — RNA stability in bone marrow samples stored in PAXgene Bone Marrow RNA Tubes refrigerated and at room temperature

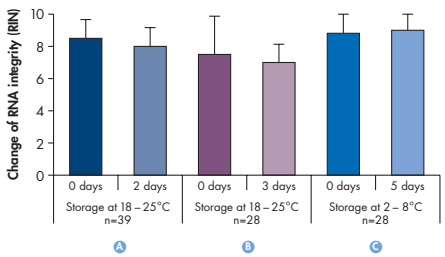

Figure 2 In three substudies (A, B, C) paired bone marrow samples were collected in PAXgene Bone Marrow RNA Tubes. A total of 95 samples were used for RNA stability under refrigerated and at room temperature conditions. RNA was purified from pairs of samples using the PAXgene Bone Marrow RNA Kit at day 0 and after A 2 days (n=39), B 3 days storage at room temperature (n=28), or C 5 days storage refrigerated (n=28). RNA integrity was evaluated using the RNA integrity numbers (RIN). Means and standard deviations of RIN are given for all samples. The RIN did not change significantly during storage of tubes for up to 3 days at room temperature and 5 days refrigerated, indicating that the RNA remains stable in the sample.

5. Results — RNA stability in bone marrow samples stored refrigerated in PAXgene Bone Marrow RNA Tubes and after freeze–thaw cycles of tubes

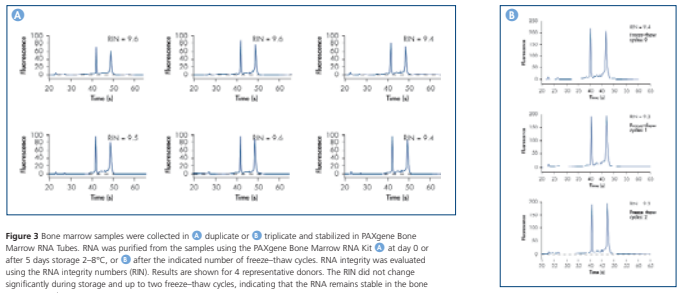

Figure 3 Bone marrow samples were collected in A duplicate or B triplicate and stabilized in PAXgene Bone Marrow RNA Tubes. RNA was purified from the samples using the PAXgene Bone Marrow RNA Kit A at day 0 or after 5 days storage 2–8°C, or B after the indicated number of freeze–thaw cycles. RNA integrity was evaluated using the RNA integrity numbers (RIN). Results are shown for 4 representative donors. The RIN did not change significantly during storage and up to two freeze–thaw cycles, indicating that the RNA remains stable in the bone marrow sample.

6. Results — RNA yields from PAXgene Bone Marrow samples

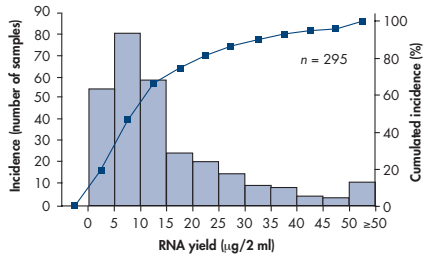

Figure 4 Bone marrow samples were stabilized in PAXgene Bone Marrow RNA Tubes, and RNA was purified using the PAXgene Bone Marrow RNA Kit. Yields are indicated for 295 samples, processed without any failures in RNA preparation (no membrane clotting, no sample loss). The median yield was 11.0 µg RNA per 2 ml sample (mean=15.8, SD=16.8, 25–75% quartiles=5.7–20.0, 5–95% quantiles=2.0–44.7 µg/2 ml).

7. Results — RNA purity from PAXgene Bone Marrow samples

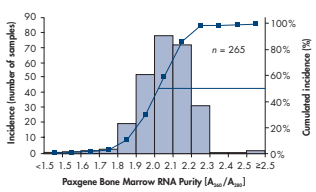

Figure 5 RNA purity (A_{260}/A_{280}) for the samples in Figure 4 were analyzed using UV spectroscopy. Purities are indicated for 265 samples. The median purity was 2.1 (mean=2.06, SD=0.15, 25–75% quartiles=1.98–2.15, 5–95% quantiles=1.83–2.23 A_{260}/A_{280}). Note that 30 of a total 295 samples analyzed for yield (Figure 4) were not analyzed for purity.

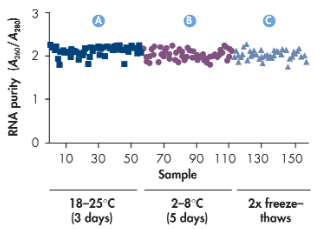

Figure 6 RNA purity for sample subset (n=157) from Figure 5. Purities are shown for RNA samples from PAXgene Bone Marrow RNA Tubes stored A for 3 days at 18–25°C (n=56), B for 5 days at 2–8°C (n=56), or C subjected to 2 freeze–thaw cycles (n=45) prior to RNA preparation.

8. Results — Residual gDNA from PAXgene Bone Marrow samples

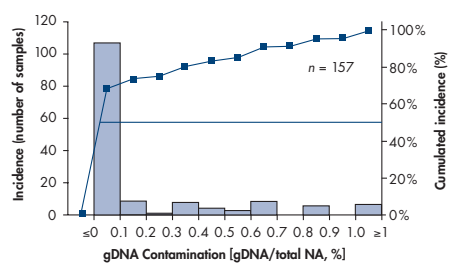

Figure 7 Residual genomic DNA (gDNA) for samples from Figure 6. Relative amounts of gDNA are indicated as gDNA per total nucleic acids. The median residual gDNA for 157 samples was 0.02% (w/w), (mean=0.25, SD=0.66, 25–75% quartiles=0.01–0.29, 5–95% quantiles = 0.00–0.85% gDNA/total NA [w/w]).

9. Conclusions

The study presented demonstrates that the new PAXgene Bone Marrow RNA System (RUO) shows high performance for the collection, storage, and transport of human bone marrow and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of RNA for molecular RNA downstream applications. Basic performance characteristics, supported by data presented here are as follows:

- For use with 2 ml human whole bone marrow
- Transcript levels stay constant and unaffected by storage as shown by high correlation of C_T values between paired samples of storage and without storage
- RNA remains stable in the sample as detected by no significant change of RIN values afer different storage times at different temperatures: 2–8°C for 5 days, 18–25°C for 3 days, –20°C and –70°C for several weeks (studies are ongoing)
- RNA integrity is unaffected by up to 2 freeze–thaw cycles of bone marrow samples stored in PAXgene Bone Marrow RNA Tubes
- Highly pure RNA is prepared from samples stored under different conditions. RNA purity is, for 90% of samples, between 1.8 and 2.2 A_{260}/A_{280}
- RNA yield is on average 11.0 µg/2 ml bone marrow
- Low genomic DNA contamination as, on median 0.02% (w/w) and for ≥95% of samples ≤0.85% (w/w) gDNA in relation to the total nucleic acid content
- RNA is applicable for downstream applications such as RT-PCR
- System is highly reliable: no sample from 295 samples showed a total failure in sample processing

The PAXgene Bone Marrow RNA System (RUO) standardizes the preanalytical steps from sample collection to stabilization and preparation of RNA. It reduces variability in preanalytical sample processing and allows better stabilization of gene transcription profiles than in unstabilized samples (e.g., bone marrow stored in EDTA tubes) making it highly valuable for molecular analysis of RNA. **For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.**

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