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Improved pre-analytical process for RNA isolation from whole blood samples

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Molecular diagnostics is becoming an important diagnostic tool in clinical hematology and oncology. Procedures for conventional PCR analyses from DNA have therefore been implemented to a great extent in hospital laboratories as routine analyses. However, trends in molecular diagnostics rely on quantitative PCR analyses using mRNA, for example in the quantification of the BCR-ABL fusion gene in chronic myelogenous leukemia patients to detect residual disease (1). RNA isolation procedures are still difficult to implement in non-academic laboratories, because of the rapid decay of RNA in standard EDTA blood tubes and logistic hurdles. Novel blood collection tubes containing a stabilizing additive have been developed as a solution to these problems (2).

We compared protocols for RNA isolation from these novel PAXgene blood collection tubes (PreAnalytix (Qiagen/BD)) and standard K_2-EDTA tubes. Additionally, we investigated the effects of storage conditions (time and temperature) for blood tubes and evaluated RNA yield and quality. To meet the requirements for further qPCR analyses, we chose a minimally required RNA concentration of 100 ng/µl.

Methods

Whole blood was drawn from 3 healthy volunteers (duplicates per volunteer; separate blood tubes per experimental condition) and patients with a hematological malignancy (n = 15) in PAXgene as well as K_2-EDTA tubes. RNA was extracted at baseline (healthy volunteers and patients) and after storage of blood samples for up to 5 days at 4°C and RT (healthy volunteers only), using the PAXgene Blood RNA kit (Qiagen) and the QIAamp RNA blood mini kit (Qiagen), including DNAse treatment and elution in 20 µl of water. RNA concentration and quality were determined spectrophotometrically (BioDrop, Isogen). Additionally, complete blood counts were measured in all subjects to correct RNA yield for leukocyte count, resulting in values expressed as ng per leukocyte. This normalization was performed since starting volumes of blood differed between the two procedures (1.25 ml for EDTA vs. 2.5 ml for PAXgene).

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Results

Striking differences were found in RNA yield when different RNA isolation procedures and storage conditions were compared (Figure 1). Overall, RNA concentrations of the eluate were remarkably higher when PAXgene tubes were used and always met the minimally required value (100 ng/µl) for qPCR analyses (Figure 1A). In addition, PAXgene tubes enabled storage at RT for up to 120 h, without compromising in RNA concentrations of the eluate. By comparison, EDTA tubes could be stored at 4°C for up to 24 h, but RNA concentrations were not always adequate for further qPCR analyses.

To compare both isolation procedures in a standardized manner, we also expressed yields as ng RNA per leukocyte (Figure 1B). We observed similar results for both isolation procedures at baseline (Figure 1B, t = 0 h). RNA yields from EDTA tubes declined drastically with storage at RT, already after 4h. Storage of tubes at 4°C for up to 24 h, however, resulted in similar results for EDTA and PAXgene tubes. Most striking differences were observed after prolonged storage at RT and/or 4°C. Only PAXgene tubes were suitable for these storage conditions.

The PAXgene RNA isolation procedure was also evaluated in our routine logistic process in which blood samples of patients with a hematological malignancy were collected and RNA isolation was carried out once a week. RNA was isolated from a total of 15 patients, resulting in a mean concentration of 196 ± 81 ng/µl and adequate yields from ±93% of the samples. Experience in sample handling might even improve this number.

Discussion

The protocols for RNA isolation and different storage conditions, as compared in our study, resulted in differences in yield that may have implications for implementation in non-academic laboratories. For RNA isolation, we strove for a production frequency of once a week. Storage of blood tubes for up to 120 h at either RT or 4°C is therefore relevant to our laboratory and can be reached by using PAXgene tubes, as indicated by our study.

Differences in observed yields for the different isolation procedures can be divided in two categories: differences observed through storage at RT and/or 4°C and differences that can be attributed to a different blood starting volume for the two investigated proto-

cols. The stabilizing agent that is present in PAXgene tubes clearly has a positive influence on RNA yields after prolonged storage (120 h). The difference in starting volume (1.25 ml for EDTA vs. 2.5 ml for PAXgene), however, plays a major role in interpreting the observed differences in yields, as shown in Figure 1B. PAXgene tubes contain a solution (7.5 ml) that preserves RNA and enables higher leukocyte loading on the clean up columns, by removing access debris that would otherwise block the columns.

Recent studies have also investigated different methods for RNA isolation from dedicated blood collection tubes (i.e. PAXgene, Tempus (Life Technologies, Thermo Fisher Scientific), and LeukoLOCK (Life Technologies, Thermo Fisher Scientific)) (3). They concluded that the PAXgene isolation procedure resulted in high quality RNA, but was more time consuming than the other methods and resulted in slightly lower yields. Tempus and LeukoLOCK tubes performed similarly and were preferred over PAXgene. However, in our opinion, LeukoLOCK tubes are not desired, as they require additional pre-analytical handling before storage of the samples. Costs of Tempus tubes and RNA isolation kits are higher than costs of material required for the PAXgene procedure, which endorses our choice for PAXgene tubes.

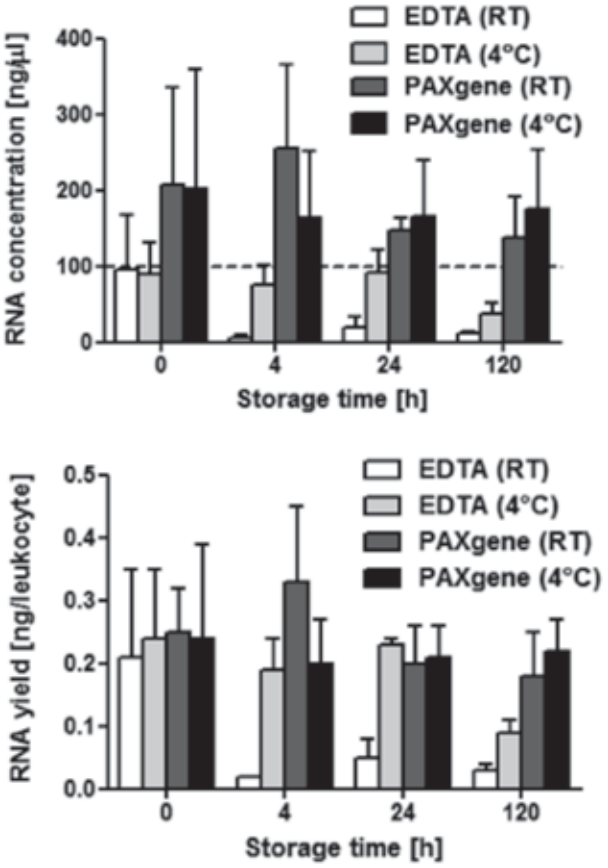

Figure 1. RNA yield after isolation procedures from EDTA and PAXgene whole blood. Means and standard deviations of RNA concentration in the eluate are shown [A] as well as RNA yield of the same measurements in ng per leukocyte [B]. Columns correspond to different storage conditions of the blood tubes; the dotted line represents the minimally required RNA concentration for qPCR analysis.

Conclusions

A higher quality pre-analytical process for RNA isolation was achieved in our laboratory when PAXgene blood collection tubes were used, compared with standard K_2-EDTA tubes. Yields from PAXgene tubes were adequate for qPCR analyses and storage for up to 5 days at RT or 4°C was guaranteed. The higher price of these tubes compared with standard K_2-EDTA tubes may be counter balanced by a more efficient logistic process, less personnel costs, a high-quality end product and the ability for out-patient blood collection.

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