TECHNICAL NOTE

Automated RNA purification

Automated RNA purification from PAXgene® Blood RNA Tubes with the chemagic™ 360 instrument

Abstract

Transcriptome analysis from whole blood is an interesting segment of research, but also difficult. RNA quality and yields are influenced by nucleases, inappropriate temperatures, and *de novo* syntheses after sample collection and storage and have an immediate effect on gene expression analyses. The use of blood collection tubes simplifies this application enormously by stabilizing RNA during sample collection and storage. Here, we show the automated isolation of high-quality RNA with the chemagic™ Total RNA Kit H24 on the chemagic™ 360 instrument from whole blood stored in PAXgene® Blood RNA Tubes. Samples were stored for several days at room temperature and 4 °C, and high-quality RNA was obtained without any inhibition in RT-PCR assays.

Introduction

Analysis of RNA in whole blood samples opens a wide range of interesting information for many research applications, but analysis of the transcriptome can be error-prone and stressful due to RNA degradation by nucleases or heat after sample collection. High-quality RNA is a crucial factor as its stability has an immediate effect on the analysis of gene expression. Blood collection tubes simplify the collection of blood, stabilize sample storage, and simplify the isolation of high-quality RNA. One commonly used blood collection tube for subsequent RNA isolation is the PAXgene® Blood RNA Tube. In this Technical Note, we demonstrate the efficient automated isolation of high-quality RNA from PAXgene® Blood RNA Tubes using our chemagic™ Total RNA Kit H24 on the chemagic™ 360 instrument after several days of storage at different temperatures.

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Product overview

PerkinElmer chemagen developed the chemagic™ Total RNA Kit H24 to isolate RNA from 2.5 ml fresh and frozen blood stabilized in the PAXgene® Blood RNA Tube (762165, PreAnalytiX (a QIAGEN BD Biosciences company), Hombrechtikon, Switzerland) with the chemagic™ 360 instrument (Figure 1).

The chemagic™ Total RNA Kit H24 kit is based on chemagen technology using M-PVA Magnetic Beads for the isolation of RNA. The RNA binds to paramagnetic beads, which are magnetically separated from the sample material. During subsequent steps, contaminants are removed, DNA is digested, and the purified RNA is transferred into an elution medium. The automated sample processing by the chemagic™ 360 instrument excludes cross contamination and ensures safe handling of infectious sample material.

For more details about the technology, visit **https://chemagen.com/technology/**

The developed kit is available in a high throughput format:

Figure 1 chemagic™ 360 instrument equipped with the chemagic™ 24 Rod Head is used for the automated extraction of RNA from blood stored in PAXgene® Blood RNA Tubes.

Product Name	Kit Name	chemagic™ Rod Head	Format	Preps/Kit	Sample Volume	Hands-on time
CMG-1084	chemagic™ Total RNA Kit H24	24	24-well	240	9 ml (incl. 2.5 ml blood)	33 min incl. centrifugation

* After thawing of samples.

Materials and Methods

2.5 ml blood of five donors was drawn into PAXgene® Blood RNA Tubes. The collection tubes were stored for up to three days at room temperature and for up to 14 days at 4 °C. Afterwards, the PAXgene® Blood RNA Tubes were stored at -80 °C. For RNA isolation, the collection tubes were thawed for 2 h at room temperature. Cell pellets were prepared by centrifugation of the PAXgene® Blood RNA Tubes (10 min, 2500 - 3500 x g), resuspension of the cell pellets in 4 ml RNase free water and a second subsequent centrifugation step. The pellets were dissolved in 2 ml ultrapure water prior to the automated RNA extraction on a chemagic™ 360 with the chemagic™ Total RNA Kit H24.

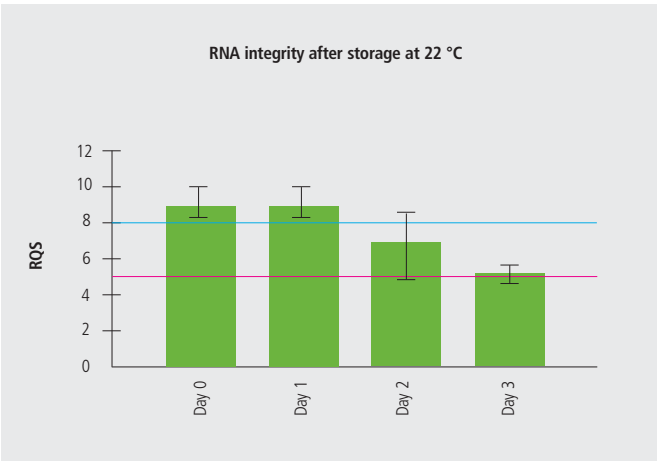
The RNA quantity and purity were determined with UV measurement with an Epoch™ Microplate Spectrophotometer (BioTek Instruments, Winooski, Vermont, USA). In addition, the concentrations and yields were measured with the Qubit™ RNA BR Assay Kit (Q10210, Thermo Fisher Scientific™, Waltham, Massachusetts, USA). The RNA integrity (RNA quality score, RQS) was measured with the RNA Assay Reagent Kit (CLS960010, PerkinElmer, Hopkinton, Massachusetts, USA) and LabChip® DNA 5K/RNA/CZE (760435, PerkinElmer, Hopkinton, Massachusetts, USA) run on a LabChip® GX Touch HT (PerkinElmer, Hopkinton, Massachusetts, USA). Reverse transcription (RT) of the purified RNA into cDNA was performed with the First Strand cDNA Synthesis Kit (#K1612, Life Technologies, Darmstadt, Germany) following the manufacturer's instructions. To avoid cDNA synthesis of fragmented mRNA without a Poly(A) tail, only the poly(T) primers were used for the cDNA synthesis. Following cDNA synthesis, quantitative real-time PCR was performed with the Maxima SYBR® green/ROX qPCR Master Mix (#K0223, Thermo Fisher Scientific™, Waltham, Massachusetts, USA) on a QuantStudio™ 5 (A34322, Thermo Fisher Scientific™, Waltham, Massachusetts, USA). The following five primer pairs were used for quantitative real-time PCR (Table 1). Primers were synthesized by biomers.net (Ulm, Germany).

Name	Gene and accession No.	Primer sequence (3‘ à 5‘)	Amplicon length
IL8	Chemokine ligand 8 NM_000584.3	IL8_fw: GGAAGGAACCATCTCACTGTG IL8_rv: GGAGTATGTCTTTATGCACTGAC	151 bp
IL1B	Interleukin 1, beta NM_000576.2	IL1B_fw: AAACCTCTTCGAGGCACAAGG IL1B_rv: GTCCTGGAAGGAGCACTTCATC	198 bp
FOS	FBJ murine osteosarcoma viral oncogene homolog NM_005252.3	FOS_fw: TCAACGCGCAGGACTTCTGC FOS_rv: TCTCCGCTTGGAGTGTATCAGTC	375 bp
FOXP3	Forkhead box P3 NM_014009.3	FOXP3_fw: AACAGCACATTCCCAGAGTTCC FOXP3_rv: GGATGGCGTTCTTCCAGGTGG	205 bp
TNFRSF10C	Tumor necrosis factor receptor superfamily member 10c NM_003841.3	TNFRSF10C_fw: ATCCCCAAGACCCTAAAGTTCG TNFRSF10C_rv: GAGATCCTGCTGGACACTCCTC	163 bp

Results

The concentrations and yields are highly dependent on the donor. Yields obtained with five different donors varied between 1 - 7 µg in Qubit and UV-measurement for samples stored at room temperature for up to 3 days and between 1-7 µg for samples stored at 4 °C for up to 14 days. In general Qubit yields were slightly lower than UV measured yields.

The RQS showed good results over the investigated time periods for both storage variants either at 4 °C or at 22 °C (room temperature). According to Fleige et al. [1] an RNA integrity above five is considered as good, while an RNA integrity above eight shows perfect RNA quality.



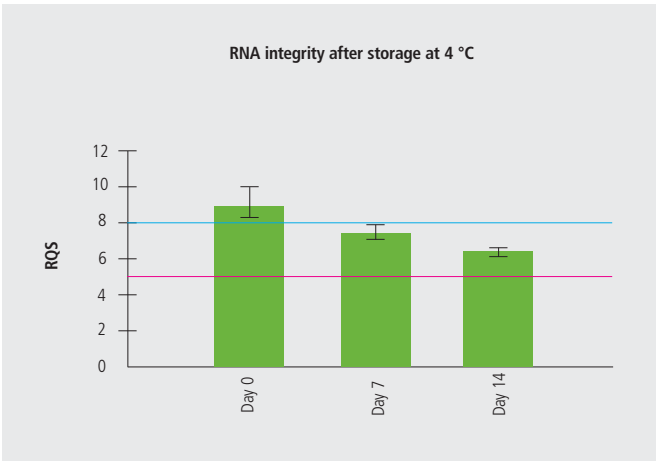


Figure 2 Appropriate RNA integrity for five donors obtained from PAXgene® RNA Tubes after storage at room temperature (left) and 4 °C (right). High-quality RNA was isolated day 0 and day 1 during storage at 22 °C and decreased during further incubation. Day 3 shows a drop in RQS value, but it is still in the acceptable range given from Feige et al. [1], although the maximum recommended storage time at RT from 72 h of manufacturer was exceeded. Good RNA integrity could be obtained from samples stored at 4°C up to 14 days.

To conserve the transcription levels of genes, it is essential to stabilize the RNA after blood draw. Therefore, we tested the stability of five different genes after cDNA synthesis via qPCR (FOS, FOXP3, IL1B, IL8 and TNFRSF10C). All qPCRs resulted in comparable data. As an example, the data sets for IL8 are shown in Figure 3 to 5.

For better comparison of qPCR results, the mean values of all donors extracted with PAXgene® Blood RNA Tubes compared with one competitor tube is shown for IL8 after incubation for up to three days at room temperature in Figure 3. In Figure 4, detailed data for PAXgene® Blood RNA Tubes are given.

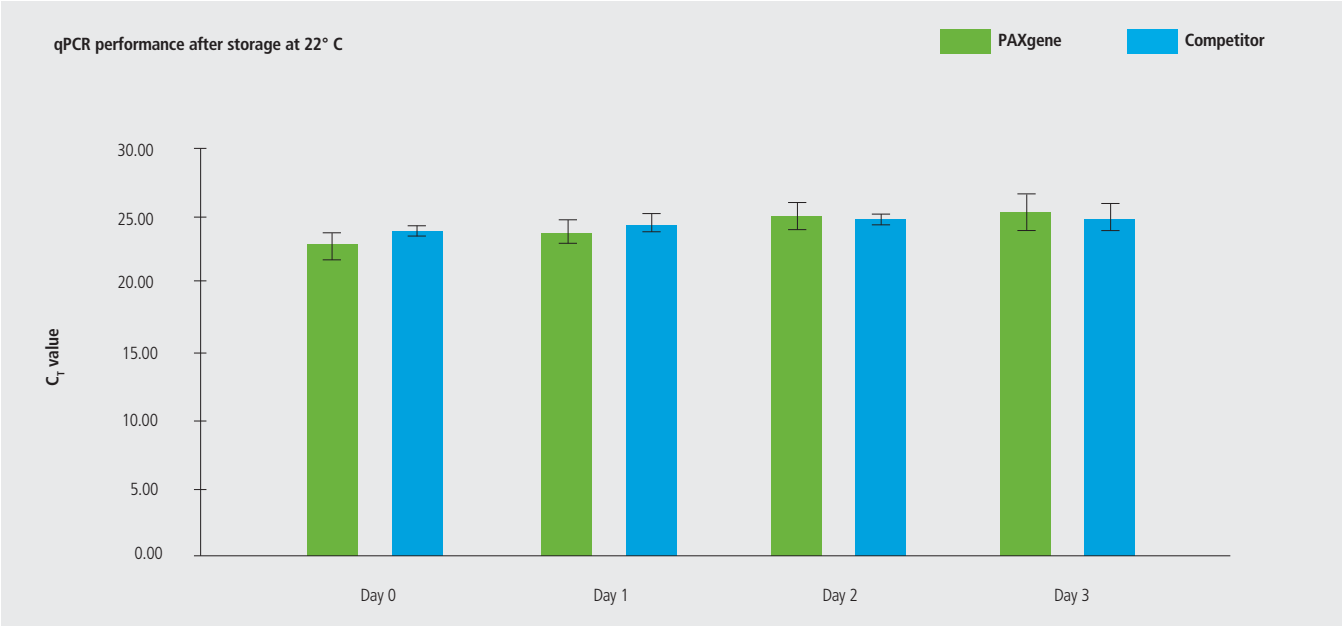


Figure 3 Comparison of IL8 primer set qPCR performance of RNA isolated from Paxgene® Blood RNA Tubes and a competitor blood collection. The isolated RNA from PAXgene® Blood RNA Tubes demonstrate similar performance compared to the competitor tube over three days of incubation at room temperature. The C_T values were stable over the complete incubation time.

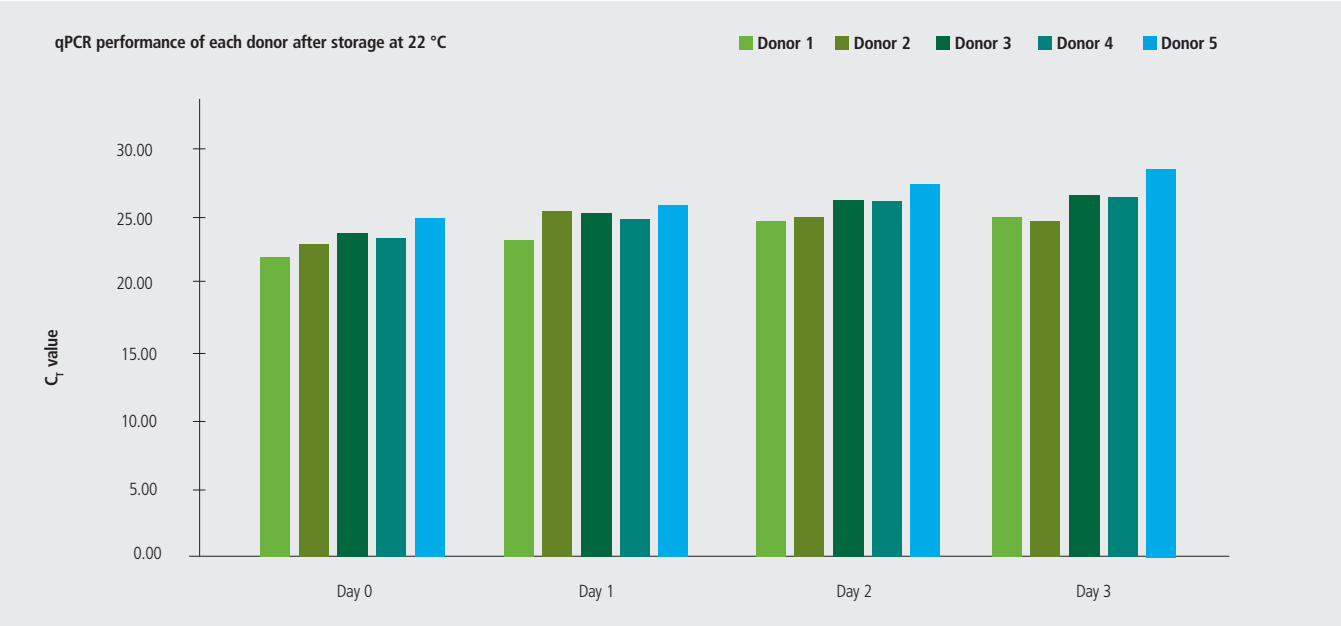


Figure 4 Reliable qPCR performance after storage at 22 °C with five different donors. Consistent C_T values were obtained from isolated RNA over the complete measured time.

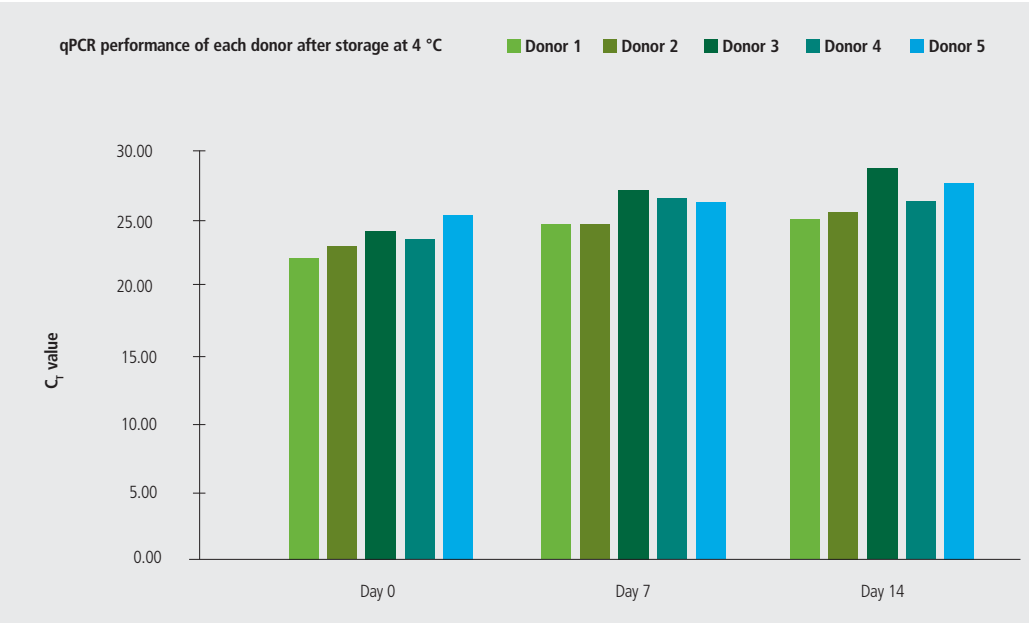


Figure 5 Consistent qPCR performance for the five single donors over 14 days of incubation at 4 °C. Only slight C_T value differences were observed from isolated RNA over 14 days of storage time, showing an extremely stable conservation of RNA collected with the PAXgene® Blood RNA Tubes and extracted with the chemagic™ Total RNA Kit H24 (CMG-1084) on the chemagic™ 360 instrument.

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Discussion

RNA yields obtained with the five donors were in a reasonable range compared to published data [2, 3]. One of the donors showed a rather low RNA yield of 1 µg. A reason for this is most likely a donor-specific variation in the white blood cell (WBC) count, which influences the total RNA content [4]. The RQS for both sample types stored at 22 °C showed good results when extracted within 72 h [5]. As expected, on incubation day 3 (~84 h after blood draw), the RQS was decreased compared to day 0, 1 and 2. The RNA integrity was extremely well maintained when storing the PAXgene® Blood RNA Tubes over 14 days at 4 °C in combination with an extraction using the chemagic™ Total RNA Kit H24. This is despite the fact that the manufacturer only recommends incubation of the PAXgene® Blood RNA Tubes for up to five days at 4 °C [5]. The stabilization of RNA levels after blood draw was shown with five different primer sets in RT-qPCR.

The C_T values reached with the RNA eluates from PAXgene® Blood RNA Tubes are comparable with the eluates from a competitor tube, both extracted with chemagic™ kits on the chemagic™ 360 instrument. Even though the RQS of day 3 was decreased compared to the previous time points, the C_T values remained constant. Moreover, the transcription levels were perfectly stable after storage at 4 °C for 14 days. In conclusion, the PAXgene® Blood RNA Tubes are one of the most used RNA-stabilizing blood collection tubes [6, 7] and they performed well in all common downstream applications with the automated isolation procedure of chemagic™ Total RNA Kit H24 on the chemagic™ 360 instrument.

PerkinElmer chemagen also provides further solutions for RNA isolation from RNA blood collection tubes such as the chemagic™ RNA Blood 9k Kit H24 (CMG-1084-S) or the chemagic™ Total RNA Kit H12 [7] to be used on the chemagic™ 360 instrument. Automated RNA purification from tissue or white blood cell pellets can also be performed with the chemagic™ RNA Tissue 10mg Kit H96 on the chemagic™ 360 instrument equipped with the chemagic™ 96 Rod Head for up to 96 samples per run.

Literature

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