Stabilization of more than 47.000 transcripts by the SARSTEDT S-Monovette® RNA Exact

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Abstract

In this application note SARSTEDT shows the ability of the S-Monovette® RNA Exact to stabilize the expression of RNA in whole blood samples. Blood samples were stored at 22.5 °C for up to 3 days and RNA levels were evaluated using the HumanHT-12 v4 BeadChip (Illumina Inc.). This microarray chip analyses the expression levels of more than 47.000 transcripts, covering more than 31.000 genes (NCBI RefSeq Release 38). The S-Monovette® RNA Exact efficiently stabilizes the levels of all investigated transcripts.

Introduction

Transcriptome analyses yield valuable information for research as well as for clinical applications such as diagnostics, disease monitoring and therapy surveillance. Patient mRNA can be obtained quickly and easily from whole blood samples. However, RNA based diagnostics are complicated due to the fact that RNA levels are highly instable in whole blood samples. RNA might either be degraded by nucleases or high temperatures [1, 2] or *de novo* synthesis of RNA might be induced after blood collection [3]. To address this issue, SARSTEDT has developed the S-Monovette® RNA Exact. This novel device contains a solution to lyse blood cells immediately after phlebotomy und efficiently inhibits degradation as well as *de novo* synthesis of RNA. Currently available RNA stabilizing blood collection devices partly show limitations regarding their RNA stabilizing capacity [4]. In this application note, SARSTEDT demonstrates the performance of the S-Monovette® RNA Exact to stabilize total RNA levels using the HumanHT-12 v4 BeadChip (Illumina San Diego, USA).

Materials & Methods

Blood Collection & Storage

Blood from three healthy donors was drawn into S-Monovette® RNA Exact blood collection tubes (Cat. No.: 01.2048.001, SARSTEDT AG & Co., Nümbrecht, Germany) or K3-EDTA tubes (04.1917.001) and samples were stored at room temperature (22.5 °C) for 0, 1 or 3 days. After incubation, samples were frozen at -80 °C until isolation of RNA.

RNA isolation

After thawing, RNA was isolated from the blood samples using the High Pure Viral Nucleic Acid Large Volume Kit (Roche Mannheim, Germany, # 05114403001). The manufacturer's protocol was slightly modified: Blood samples were treated with Proteinase K only, not with binding buffer or carrier RNA, and incubated for 15 min at room temperature instead of 70 °C. The isolated RNA was treated with DNase (Thermo Scientific, # EN0521) after elution according to the manufacturer's instructions.

RNA integrity number (RIN)

RIN was measured with a Bioanalyzer 2100 (Agilent, Waldbronn, Germany) using RNA Nano 6000 Chips (Agilent).

Analysis of gene expression

Gene expression was assayed with the HumanHT-12 v4 BeadChip (# BD-103-0204, Illumina San Diego, USA). This microarray analyses the expression levels of more than 47.000 transcripts, covering more than 31.000 genes (NCBI RefSeq Release 38). Gene expression data were analyzed using the software R (V3.0.2) and the Bioconductor Package "limma". The measurements and subsequent data analysis were carried out by Life & Brain GmbH (Bonn, Germany).

Results

To evaluate the quality of the RNA isolated from whole blood samples, the RIN was determined. All samples showed a RIN>7 indicating a good RNA quality (data not shown) [5]. No obvious differences were detected between RNA from EDTA anticoagulated whole blood and from blood stored in the S-Monovette® RNA Exact. To this end, all samples were included in the subsequent microarray experimental analyses.

The internal control probes on the microarray showed no irregularities, a homogenous intensity distribution and comparable numbers of the detected transcripts (data not shown). The obtained results of the expression analysis of both EDTA and RNA Exact stored samples

were referred to the same sample (day 0). The analysis of the hybridization compares the signals between day 0 and samples stored for 1 and 3 days. Genes with an expression level differing more than factor 2 (=doubling or halving of the expression level) were counted as differentially expressed.

For the cluster analysis 199 variable transcripts were chosen (coefficient of variation, CV, between 0.5 and 10). Two distinct clusters were found. Cluster 1 contained all EDTA anticoagulated samples which clustered according to storage time rather than donors. Cluster 2 contained the day 0 sample and all samples stored in the S-Monovette® RNA Exact. These samples clustered according to donors rather than storage time (Figure 1).

In non-stabilized, EDTA anticoagulated samples there was a linear increase of differentially expressed transcripts over time. The number of differentially expressed transcripts between day 0 and day 3 (n=768) was almost equal to the number of differentially expressed transcripts between day 3 of the EDTA anticoagulated samples and day 3 of the RNA Exact stored samples (n=777). There were no differentially expressed transcripts between day 0 and day 3 in samples stored in the S-Monovette® RNA Exact (Figure 2).

Discussion

Within samples stored in the S-Monovette® RNA Exact, transcript levels were efficiently conserved. The non-stabilized, EDTA anticoagulated samples clustered according to storage time rather than donor. This means that there are differences in the amount of transcripts between day 0, day 1 and day 3 and there is only minor variation between the investigated donors. The group analysis confirms the aforementioned findings of many transcripts being differentially present in non-stabilized, EDTA anticoagulated samples. Taken together, non-stabilized, EDTA anticoagulated samples are influenced by the storage time. Samples stored in the S-Monovette® RNA Exact and the day 0 samples clustered according to donors rather than incubation time. This shows that any changes in the transcript pattern over the investigated time are rather due to donor variations than to the quality differences caused by the storage time. The group analysis confirms that RNA Exact stabilized samples do not show differentially present transcripts between the investigated time points (Figure 1). These findings demonstrate that the expression levels of all 47.000 investigated transcripts are efficiently stabilized in the S-Monovette® RNA Exact for at least three days at room temperature.

The correlation matrix illustrates how many genes are differentially expressed between the samples. In non-stabilized, EDTA anticoagulated samples the expression levels of several hundred transcripts changed between day 0 and day 3. Interestingly, the sum of the differentially expressed transcripts between day 0 and day 1 as well as between day 1 and day 3 is not equal to the number of differentially expressed transcripts between day 0 and day 3. This is due to the fact that the detection limit requires to be exceeded only once between day 0 and day 1 rather than twice between day 0 and 3. There are no changes in transcript levels of samples stored in the S-Monovette® RNA Exact.

Taken together, these data show that the S-Monovette® RNA Exact stabilizes the expression levels of more than 47.000 transcripts in whole blood samples for at least three days.

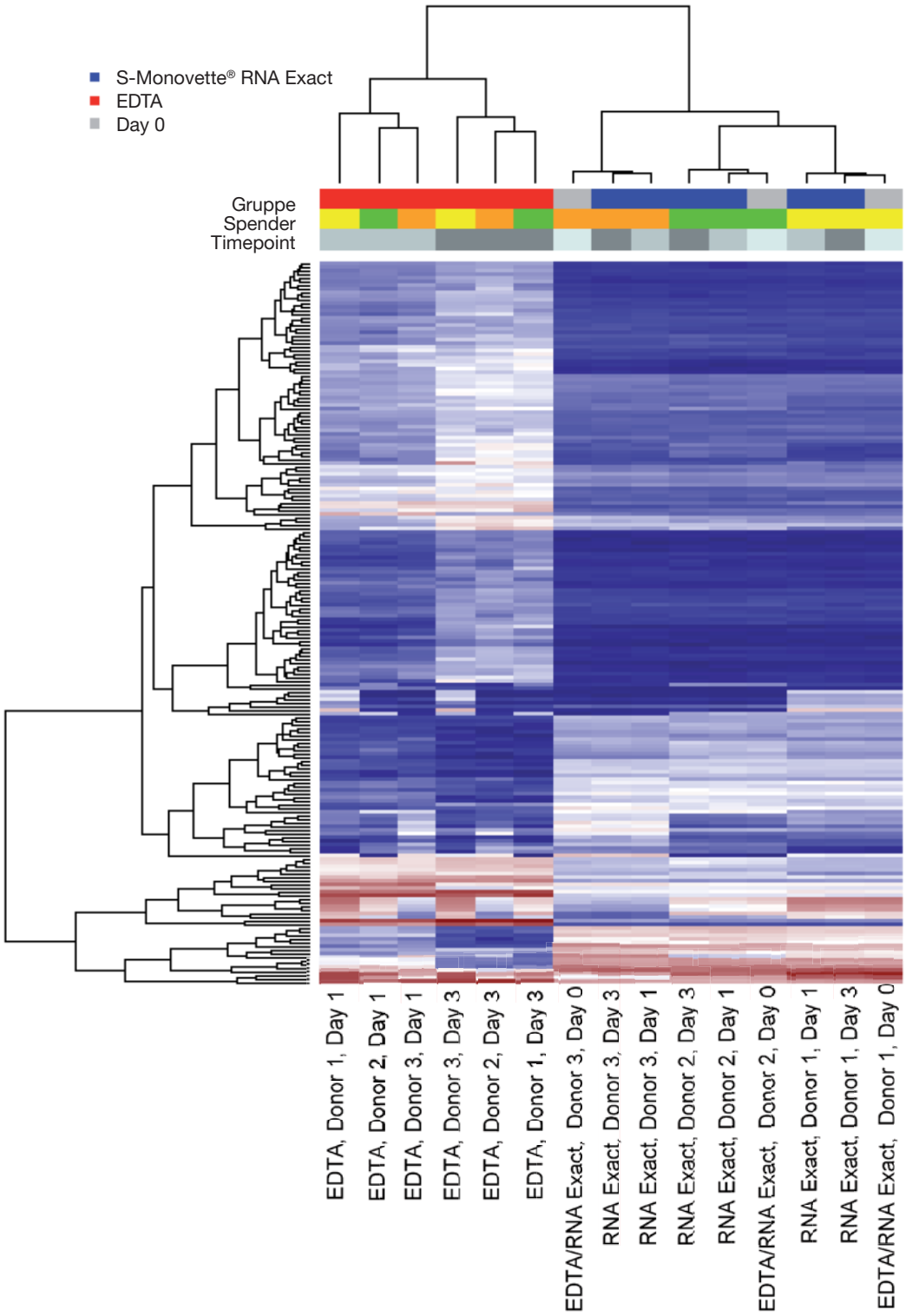

Figure 1: Cluster analysis of the data derived from the HumanHT-12 v4 BeadChip

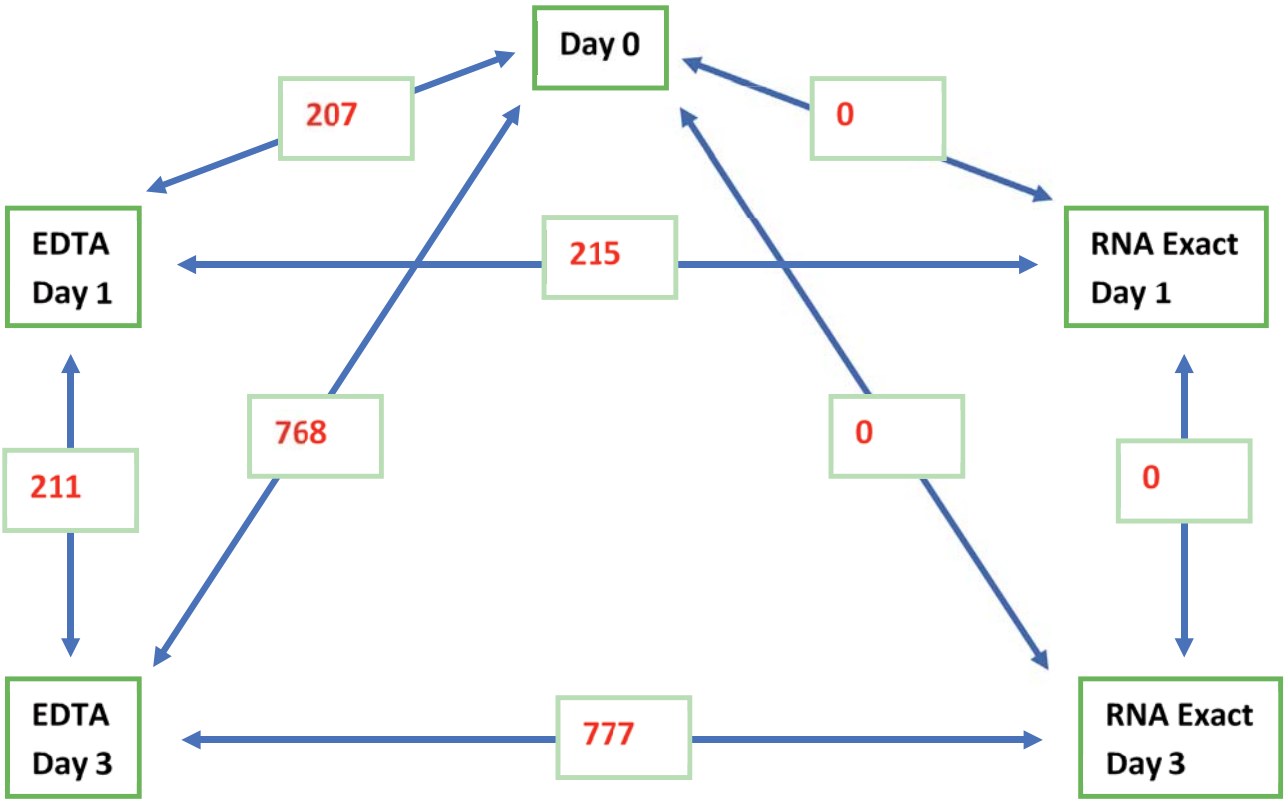

Figure 1: Cluster analysis of the data derived from the HumanHT-12 v4 BeadChip
The numbers of genes that are differentially expressed between the indicated time points are displayed. Day 0: EDTA/RNA Exact samples on day 0; KT1: EDTA-samples stored for 1 day; KT3: EDTA-samples stored for 3 days; T1: RNA Exact-samples stored for 1 day; T3: RNA Exact-samples stored for 3 days.

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