Genome-wide comparison of two RNA-stabilizing reagents for transcriptional profiling of peripheral blood

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Peripheral whole blood is relatively easily obtained for monitoring gene expression for biomarker discovery using transcriptomic platforms such as genome-wide microarrays. However, whole blood provides challenges caused by sensitivity for ex vivo incubation and overrepresentation of globin mRNAs. We compared the performance of 2 commercial whole blood preservation methods, TEMPUS (Applied Biosystems, Foster City, CA) and PAXgene (PreAnalytiX, Qiagen BD, Valencia, CA), using 2 RNA amplification protocols and high-density microarrays. Performance of commercial globin mRNA reduction protocol also was studied. Human peripheral blood samples collected with TEMPUS and PAXgene Blood RNA tubes were amplified with the RiboAmp OA 1 Round RNA Amplification Kit (Arcturus; Applied Biosystems) and the Affymetrix (Santa Clara, CA) small sample protocol. Affymetrix globin reduction protocol was applied for total RNA samples. Samples amplified with RiboAmp were hybridized on Illumina Sentrix HumanRef-8 Expression BeadChips (Illumina Inc, San Diego, CA) and subjected to statistical analyses. RiboAmp mRNA amplification did not notably amplify globin mRNA that is overrepresented in RNA isolated by both TEMPUS and PAXgene preservation. Enzymatic depletion of globin transcript reduced the quality of total RNA and is thus not recommendable. Microarray analysis showed acceptable correlation within and between the RNA preservation methods, but altogether 443 transcripts were differentially expressed between RNA samples preserved in TEMPUS and PAXgene tubes. We demonstrated that the 2 tested blood RNA-preservation methods combined with RiboAmp mRNA amplification may be used for microarray experiments without the need for a prior globin RNA reduction. However, because genes involved in immune cell functions and gene regulatory pathways were differentially expressed as a result of the technical bias between the preservation methods, they should not be used in the same analytic setting. (Translational Research 2013;161:181–188)

Abbreviations: aRNA = amplified RNA; GSEA = gene set enrichment analysis; IPA = ingenuity pathways analysis; PBMC = peripheral blood mononuclear cell; SAM = significance analysis of microarrays

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AT A GLANCE COMMENTARY

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Background

Peripheral blood as the main transportation route of immune cells reflects the immune response of the body. Two well-established methods with RNA-stabilizing reagents are available for transcriptional profiling of peripheral blood. However, no comparison has been published in which blood from the same individuals is drawn into both collection systems in parallel.

Translational Significance

We noticed gene expression bias, associated with leukocyte cellular functions and canonical pathways, between the RNA preservation methods. Thus, different methods should not be used in the same analytic setting. We also demonstrated that mRNA amplification escapes globin RNA bias of whole blood samples, allowing more simple sample processing.

Two well-established methods, TEMPUS (Applied Biosystems, Foster City, CA) and PAXgene Blood RNA (PreAnalytiX, Qiagen BD, Valencia, CA) tubes, currently are available for *ex vivo* transcriptional profiling of human peripheral blood.[1,2] Whereas gene expression profiles of whole blood cells are normally highly sensitive for ex vivo incubation,[3] these 2 methods use RNA-stabilizing reagents included in the collection tubes to ensure that the transcription profiles obtained correctly reflect the physiologic status at the time of the blood draw.

Although the effect of the minor cell types may be masked by the predominant ones when profiling gene expression in a bulk tissue,[4] peripheral blood is the most practical tissue to profile gene expression of the human immune system in a clinical setting. Because blood is the main route for transportation of the immune cells, transcriptional activity in peripheral blood may reflect imbalances in the immune system of the patient and provide a window for monitoring activity of the entire immune system in the body. Therefore, blood transcriptome analyses with microarrays may reveal disease-related molecular pathways, genes and susceptibility loci, general autoimmune signatures shared between different autoimmune diseases, and expression signatures that allow monitoring of disease activity and response to treatment.[5] In the long-term storage, the RNA-stabilizing collection tubes are shown to yield more stable gene expression profiles compared with cryopreservation of peripheral blood mononuclear cells (PBMCs) in liquid nitrogen.[6] Thus, the systems provide feasible procedures for gene expression profiling and biomarker discovery from clinical sample material.

Both PAXgene and TEMPUS tubes yield only micrograms of total RNA. Thus, amplification of the target mRNA is required to get enough amplified (a)RNA for multiple hybridizations and validation experiments. Although multistep amplifications are laborious, and hybridization specificity and relative transcript abundance may be slightly distorted by the amplification,[7-9] the benefits of amplification are substantial. Amplification of mRNA has been proven to be repeatable and reproducible.[8,10-12] In addition, aRNA is more sensitive than the nonamplified mRNA in detecting significantly more transcripts.[8,9] Furthermore, other molecular methods have confirmed that the transcripts that were not detected in nonamplified material were actually expressed in the cells and not amplification artifacts or indicators of unspecific binding.[13] The increased sensitivity arises from a 3 to 10 times higher relative amount of labeled aRNA targets than what can be obtained using corresponding mRNA in the total RNA targets. In addition, the greatest amplifying effect appears on low copy number transcripts.[14] Even partially degraded RNA can yield amplification products for reliable measurement of relative expression levels.[15]

Several studies have assessed the quality of RNA and its applicability to gene expression microarray studies when samples collected into PAXgene or TEMPUS tubes were isolated immediately or after freezing. However, a comprehensive comparison of the 2 methods for analysis of human peripheral blood has not been addressed. In previous studies, investigators focused on 1 of the 2 methods available,[16-20] blood samples were manipulated before aliquoting into tubes with RNA-stabilizing reagents,[21] or the genome-wide expression profiles were not addressed.[22,23]

In the current study, we investigate how taking the sample to PAXgene or TEMPUS tubes influences the peripheral blood transcription profiles after RNA amplification. The results are also compared with the transcriptional profiles of PBMCs. Furthermore, we demonstrate that a relatively high amount of globin RNA is present in samples collected using either of the 2 preserving methods, but the linear amplification of RNA appears to bypass the globin problem that is detected when PAXgene samples are processed without amplification.

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