

Diagnostic and prognostic potential of miRNA profiling in human blood

In clinical research studies, blood samples are widely used for patient monitoring. The profiling of miRNA in combination with other genetic data in human blood holds much promise for the development of genetic markers of disease and can aid in the development of diagnostic and prognostic tests.

by Dr M. Kruhøffer 

Molecular genetic data become increasingly important during the process of clinical testing of new drugs. Recently, microRNAs (miRNA) were found to be involved in disease development and progression. Their

natural role in developmental processes like angiogenesis, neurogenesis and stem cell differentiation supports their involvement in cancer progression. miRNAs have been identified that have tumour suppressor and oncogene functions and expression changes of several miRNAs may have diagnostic and prognostic significance [1].

infectious diseases, nucleic acid based molecular diagnostic tests for cancer or metabolic diseases have been designed. Regulatory agencies like the American Food and Drink Authority (FDA) encourage the generation of genetic data during clinical trials to better estimate and document the effects of drugs in humans [2].

Profiling of these small regulatory RNA species may provide additional valuable information to pharmaceutical companies as well as diagnostic test developers. Besides testing for

Sampling of blood is a standard procedure in most clinical trials as well as many diagnostic testing procedures. Pathological conditions in organs and remote tissues are often detectable in gene expression profiles from blood samples.



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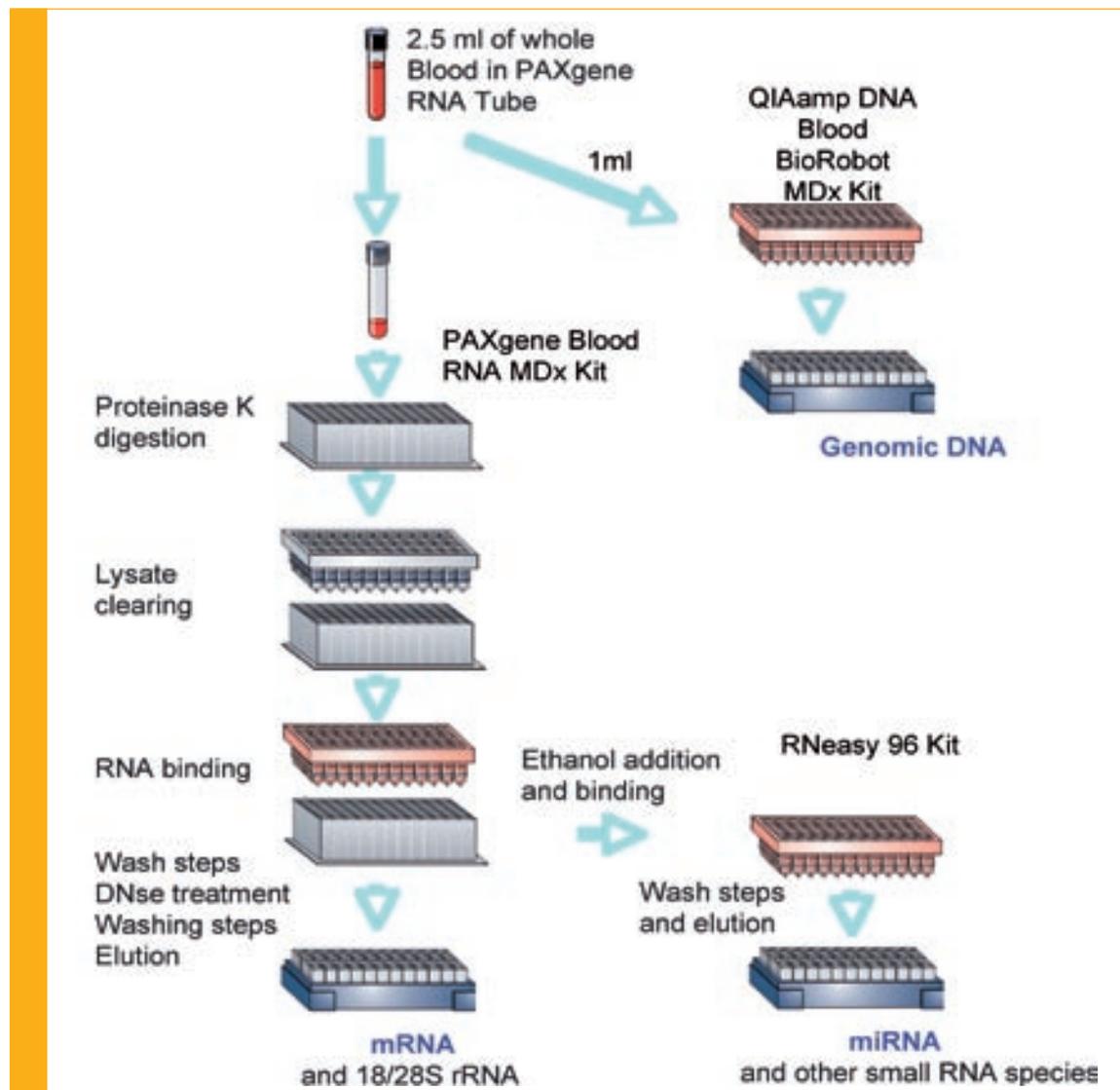


Figure 1. Tri-X starts with 2.5 mL whole blood collected in a single PAXgene Blood RNA Tube (PreAnalytiX). Genomic DNA is isolated from a 1mL aliquot with the QIAamp Blood DNA protocol (QIAGEN). The remaining sample is extracted with the PAXgene Blood RNA MDx procedure (PreAnalytiX) to isolate mRNAs and large rRNA fragments. Small RNA species including miRNA are isolated from the flow-through of the PAXgene Blood RNA MDx protocol binding step via a modified RNeasy (QIAGEN) procedure.

Thus blood may serve as a surrogate tissue that can be collected with minimal effort and inconvenience to the patient. A recently published method [3], which allows the isolation of genomic DNA, mRNA and miRNA from a single PAXgene Blood RNA Tube is shown in Figure 1.

The resulting amounts of nucleic acids from the TRI-X method are sufficient and of an adequate quality to perform microarray experiments. In contrast to classical diagnostic parameters like blood sedimentation rate (BSR) or cholesterol content, which are

stable in standard blood collection tubes, the nucleic acid based information can be affected very rapidly. Therefore the use of a collection device with the ability to stabilise the transcriptome efficiently like the PAXgene Blood RNA Tube is mandatory. It is well documented that post-phlebotomy changes in the transcription profile caused by degradation and gene induction lead to inconsistent results.

A completely automated isolation procedure would decrease failure rates and simplify high sample throughput logistics via sample tracking [Figure 2]. The TRI-X procedure is readily implemented in AROS laboratories in a GLP environment that is usually required for clinical trials.

Although miRNAs have been found in all mammalian tissues examined so far, there is little published information about miRNA expression in blood or the haemopoetic system [4,5]. Some consistency can be found if this very limited number of studies is compared [Table 1]. It is likely that miRNA expression profiles in

miRNA name	expression described by		
	Kruhøffer <i>et al.</i> ²	Georgantas <i>et al.</i> ³	Landgraf <i>et al.</i> ⁴
hsa-miR-142	whole blood		haemopoetic
hsa-miR-144	whole blood		haemopoetic
hsa-miR-202	whole blood	PBSC & BM	
hsa-miR-30b	whole blood	PBSC & BM	
hsa-miR-30d	whole blood	PBSC & BM	
hsa-miR-223	whole blood	PBSC & BM	haemopoetic
hsa-miR-25	whole blood	PBSC & BM	
hsa-miR-29c	whole blood	only in BM	
hsa-miR-23a	whole blood	PBSC & BM	
hsa-miR-20a	whole blood	PBSC & BM	
hsa-miR-150	whole blood		haemopoetic
hsa-miR-23b	whole blood	PBSC & BM	
hsa-miR-27a	whole blood	only in BM	
hsa-miR-155	whole blood		haemopoetic

Table 1. Comparison with findings in recent literature.



Figure 2. BioRobot MDx workstation (QIAGEN) for the PAXgene Blood RNA MDx Kit (PreAnalytiX) and the QIAamp DNA Blood MDx Kit (QIAGEN) as part of the TRI-X procedure.

white blood cells for blood related diseases can be defined.

In peripheral blood, transcriptional profiles for non-blood related diseases like multiple sclerosis, ischaemic stroke and colon diseases has been identified. It is therefore also feasible to assume that blood cell miRNAs can reflect the activity in other tissue compartments in the same way, and thus serve as a diagnostic and/or prognostic tool.

For example, some of the 42 miRNA species detected by Kruhøffer *et al.* [3] like let-7a, mir145 and mir195 are supposed to be connected to different forms of cancer and could potentially be used as a starting point for diagnostic or prognostic assay development. Other authors [4,5] identified miRNA species that have specific expression profiles in cells of the haemopoetic system or tumour cell lines derived from haemopoetic cells.

Beside miRNAs which are generally expressed in human blood, other miRNA species were found to be expressed only in a subgroup of donors (Kruhøffer, unpublished). It is likely that some more information with clinical relevance could be collected from these miRNA species. Overall, such profiles could help to define a golden healthy expression profile for comparison with disease profiles, which should be established from comprehensive expression profiling studies.

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