Maintaining the stability and integrity of RNA from whole blood samples

Stabilised RNA is a necessity for accurate gene expression studies. The PAXgene Blood RNA system minimises preanalytical variables and has established a new standard in RNA processing. In addition, stabilisation of RNA can now be automated on a small bench-top instrument. This system is currently being used in the development of clinical assays.

by Dr K. Guenther and Dr M. McCluskey

In some conditions, gene expression levels can be measured to indicate disease status or prognosis. In chronic myeloid leukaemia (CML), for example, the transcript level of the BCR-ABL gene can be measured using real-time PCR of RNA from peripheral whole blood samples to indicate disease status [1].

In addition to established gene expression tests such as that used for CML, research is undertaken to measure gene expression patterns for new prognostic tests. For example, a recent research paper measured cytokine RNA-expression in the TNF-alpha and IL-10 genes of severe burn patients. Trends were observed in these gene transcript levels that indicated that they may have prognostic value in order to determine patients who are likely to survive [2].

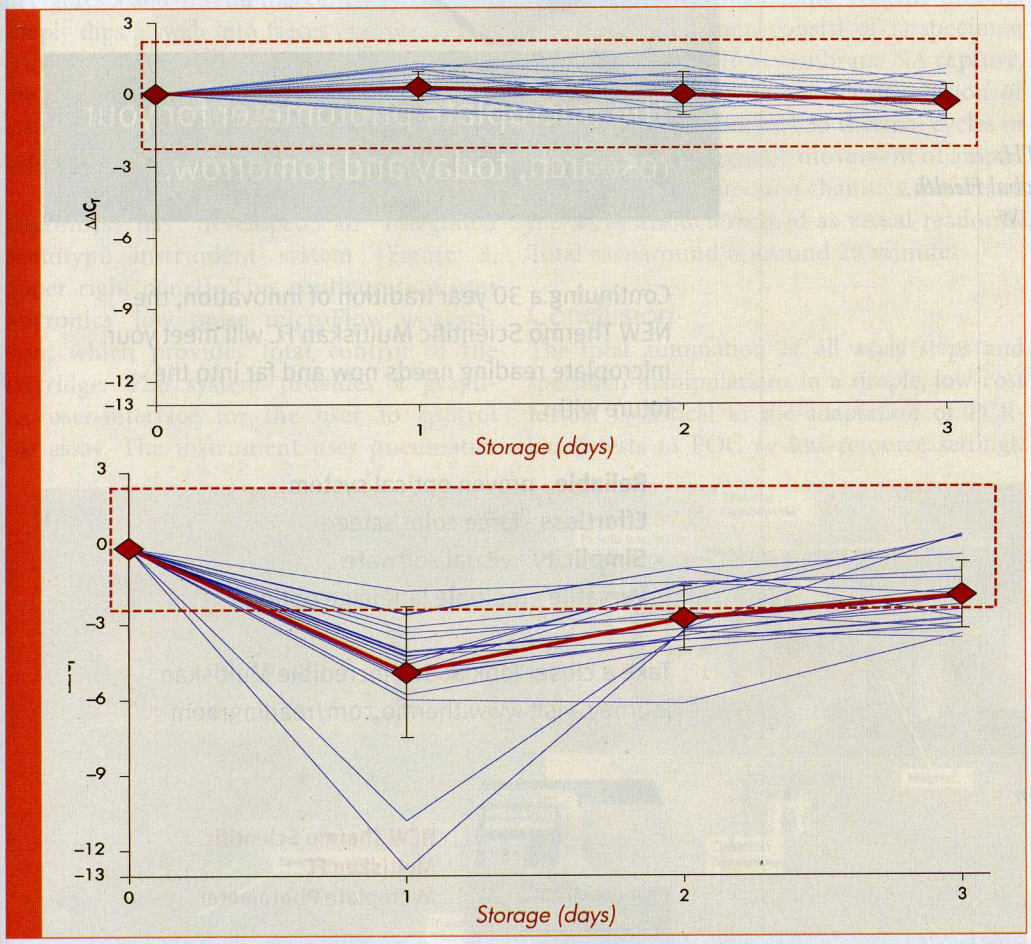

Figure 1. Blood was drawn from 10 donors, with duplicate samples, and stored at 18-25°C for the indicated number of days, followed by total RNA purification. Top graph: blood was collected and stored in PAXgene Blood RNA tubes, and total RNA was purified using the PAXgene Blood RNA kit. Bottom graph: blood was collected and stored in standard blood collection tubes with EDTA as an anticoagulant, and total RNA was purified using a standard organic-extraction method with silica-membrane-based RNA cleanup. Relative transcript levels of FOS were determined by real-time, duplex RT-PCR, using 18S rRNA as an internal standard. The values for all samples are plotted, with means and standard deviations of all samples shown. The dashed lines indicate the ±3x total precision of the assay (2.34 CT).

RNA collection and purification are crucial steps in this process. The quality and integrity of the RNA to be tested must be maintained to obtain a true and accurate transcript level. After collection, RNA transcripts in blood are often up- or down-regulated, meaning that assays from these samples do not necessarily give true results. Another factor to consider is that RNA is degraded over time, which can also affect downstream results. Down-regulation and degradation can be particularly detrimental to tests involving genes with low expression transcripts. For an accurate representation of *in vivo* RNA transcript levels, stabilisation of the whole blood sample is required at the time of collection.

Stabilised and standardised processing

It is already possible to minimise transcript changes and confidently use RNA as a diagnostic biospecimen. The PAXgene Blood RNA system (PreAnalytiX GmbH, Switzerland) standardises and simplifies the collection, immediate stabilisation and transport of human blood samples for the subsequent storage and extraction of intracellular RNA. It is the first IVD product for the collection, storage and transport of blood, and the stabilisation of intracellular RNA in a closed tube. It allows the subsequent isolation and purification of the intracellular RNA from this whole blood sample so that RT-PCR can be carried out for molecular diagnostic testing.

Performance characteristics for the system were established with FOS and IL1B gene transcripts. Users are responsible for establishing appropriate performance characteristics for other target transcripts. If stabilisation is not undertaken at point of collection, an artifact can be measured rather than the true gene-transcript level, which can lead to misleading gene expression results. The PAXgene Blood RNA tubes stabilise intracellular RNA and standardise RNA purification from the point of blood collection. Messenger RNA is stabilised for up to three days at room temperature (18-25°C) and for up to five days at 2-8°C with FOS and IL1B. Figures 1A & 1B show the difference between blood collected using this system, and blood collected

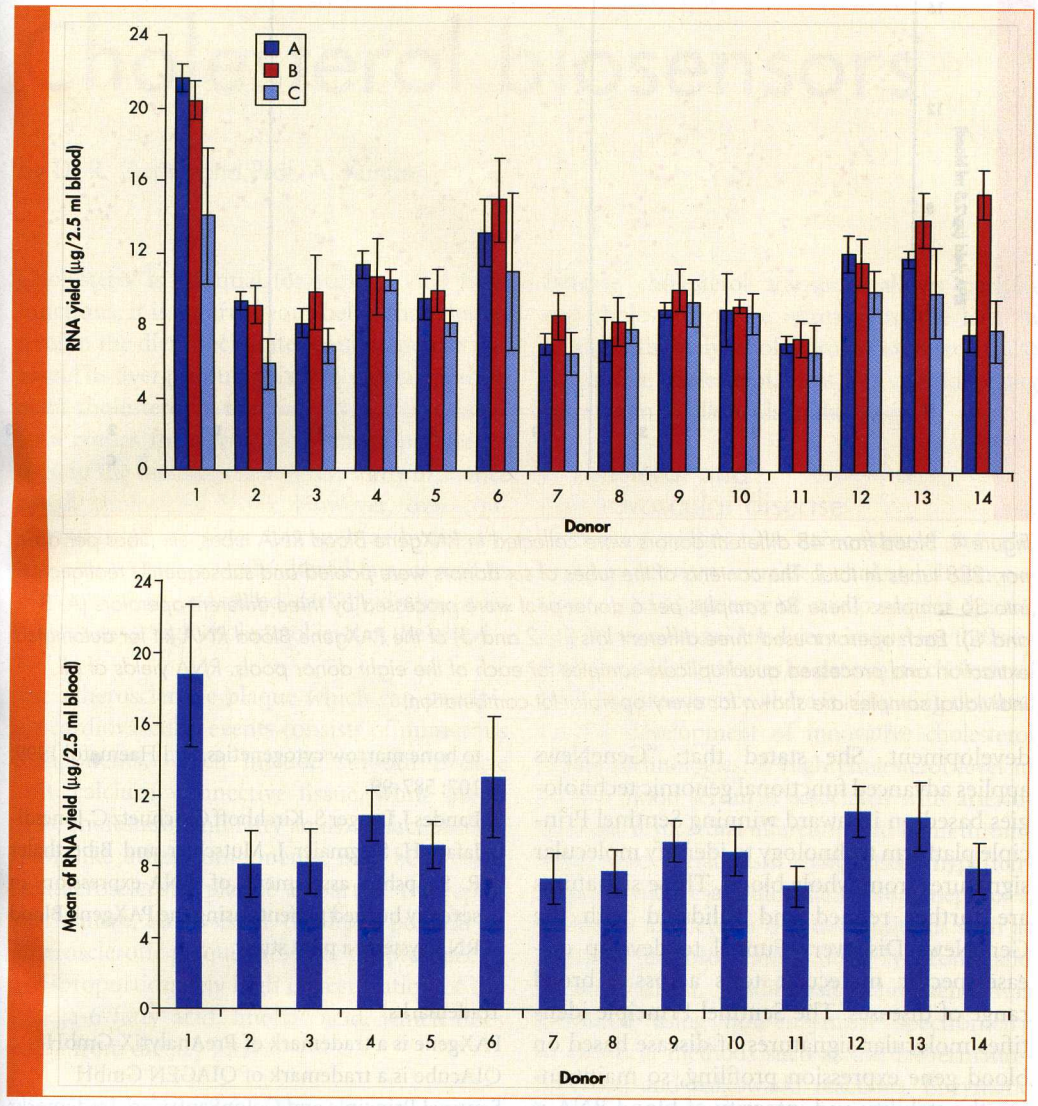

Figure 2. Reproducible and repeatable RNA purification. Quadruplicate blood samples from 14 donors were manually processed by each of three technicians (A, B and C). Three sets of equipment were used, and all samples prepared by a single technician were processed using the same equipment. Top: means and standard deviations of RNA yield per replicate samples from the same donors and different technicians are shown. Bottom: twelve replicate blood samples from each of 14 donors were processed by the three different technicians. Means and standard deviations of RNA yield per samples from the same donors and all technicians are presented. For all RNA samples, A260/A280 ratios ranged from 1.8 to 2.2.

in standard blood collection tubes, containing EDTA as the anticoagulant, at room temperature with FOS as the gene transcript of interest. This result indicates that the first step is critical for accurate downstream analysis and verifies the importance of preanalytical steps in all experimental designs.

Studies are underway by PreAnalytiX to assess how long tubes can be stored before processing after blood has been drawn. Current results indicate that stabilisation is maintained at -20 to -80°C for at least two years. The system has been used in the Biomedical, Pharmalogical, Clinical and Molecular Diagnostic research sectors. The system is manufactured by PreAnalytiX GmbH (Hombrechtikon Switzerland), a joint venture of QIAGEN (Hilden, Germany) and BD (Franklin Lakes, NJ, USA). It comprises the PAXgene Blood RNA tube and the PAXgene Blood RNA kit. The tube and manual kit, when used in combination, carry the CE label for use in Europe and FDA clearance for use in the United States.

To further increase standardisation of the RNA extraction process, a new low-throughput automated option is available. Since May 2008, the manual kit has also been offered as an automated solution in some countries in Europe (with CE label) and outside North America. To date, FDA clearance of this protocol is not yet available*.

In addition to RNA stabilisation, results with the PAXgene Blood RNA System are repeatable and reproducible. In the study described here [Figure 2], samples from fourteen donors were processed by three operators and results of RNA yield from 2.5mL of blood are given.

* Launched in Europe with CE label and pending 510(k) clearance in the United States.

From manual to automated options

Automated systems are routinely used in high-throughput molecular biology or clinical research labs, enabling scientists to benefit from the inherent advantages of automation, such as standardised processing, increased user safety when handling potentially infectious samples, more reproducible results, and increased time and labour efficiency. In low-throughput laboratories, single spin columns are most frequently used for purification of nucleic acids. Up to now, these purification procedures were not amenable to automation, preventing a huge number of researchers from enjoying the benefits mentioned above.

QIAGEN developed a small bench-top instrument, the QIAcube, that enables fully automated sample processing, from sample lysis to elution of highly pure nucleic acids using a spin column kit format [Figure 3]. A protocol for the processing of the PAXgene Blood RNA tubes in conjunction with the PAXgene Blood RNA kit (CE Version) has been developed on the QIAcube to give users without a high-throughput workload the chance to access automation. Standardised, automated processing provides results that are comparable between experiments, operators and labs, as can be seen in Figure 4. The ease-of-use of QIAcube means that the system can be operated by anyone, from the novice to the expert, making it a valuable tool for molecular biology and clinical research labs.

A customer application of the PAXgene system

Many researchers understand that to measure true gene expression transcript levels, it is important to make collection of samples and purification of biomolecules an efficient and standardised process.

Gailina Liew, Chief Operating Officer of GeneNews (Richmond Hill, Canada) has seen the benefits of using the system for discovery, as well as molecular test development. She stated that: "GeneNews applies advanced functional genomic technologies based on its award winning Sentinel Principle platform technology to identify molecular signatures from whole blood. These signatures are further refined and validated with the GeneNews Discovery Funnel to develop disease-specific molecular tests across a broad range of diseases. The Sentinel Principle identifies molecular signatures of disease based on blood gene expression profiling, so maintaining the stability and integrity of blood RNA is critical. GeneNews' scientists have extensively assessed blood sampling methods with regard to *ex vivo* changes in blood gene expression profiles and have decided to incorporate the use of the PAXgene Blood RNA system for the collection and handling of blood samples into its development and validation activities. GeneNews also uses the system as a convenient method for the collection of patient samples in a clinical setting for its ColonSentry assay, the world's first molecular blood test for colorectal cancer screening, launched commercially in Canada in July 2008. The ColonSentry test assesses an individual's current risk for colorectal cancer and is based on measuring the differential levels of RNA expression of seven genes from a whole blood sample. The use of the PAXgene system for the collection of patient blood samples is essential to ensure that the RNA remains stable and provides good quality results. The ColonSentry test will be made available in Asia, the United States and Europe in 2009-2010."

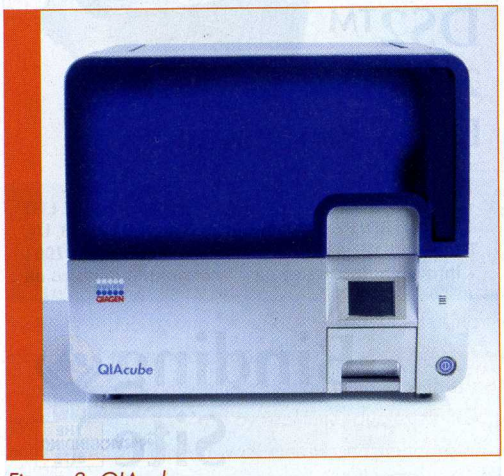

Figure 3. QIAcube.

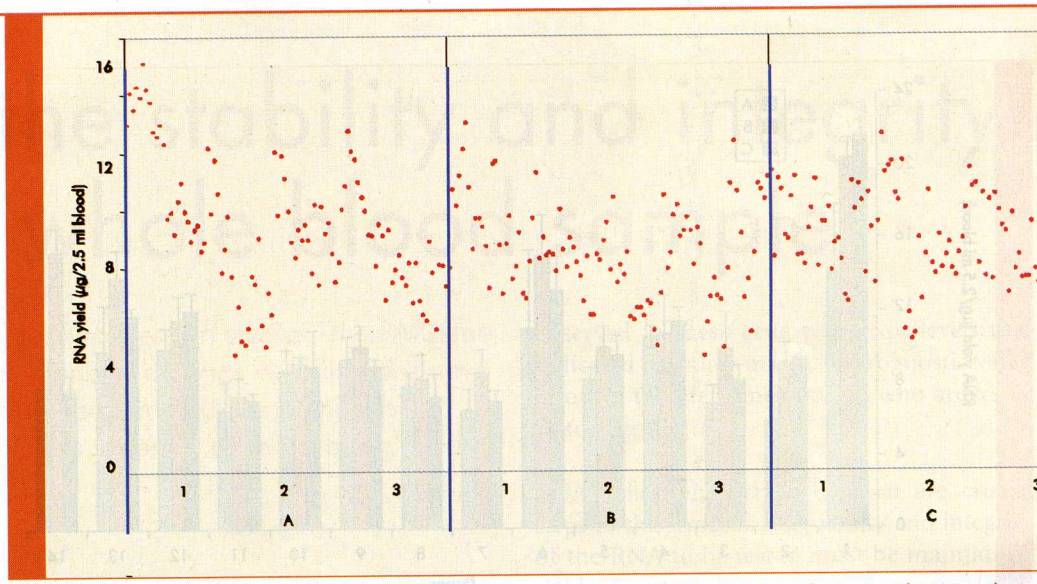

Figure 4. Blood from 48 different donors were collected in PAXgene Blood RNA tubes; six tubes per donor, 288 tubes in total. The contents of the tubes of six donors were pooled and subsequently realiquoted into 36 samples. These 36 samples per 6-donor-pool were processed by three different operators (A, B and C). Each operator used three different lots (1, 2 and 3) of the PAXgene Blood RNA kit for automated extraction and processed quadruplicate samples for each of the eight donor pools. RNA yields of all individual samples are shown for every operator-lot combination.

References

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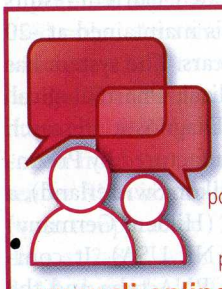

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