Automated, Magnetic Bead miRNA Enrichment from Stabilized Whole Blood: Development of an Optimized Protocol and Comparison to Established Procedures by the SPIDIA* Consortium

*Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

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Introduction

Funded by the European Union, SPIDIA is a 4-year project whose mission is to improve and standardize preanalytical procedures for *in-vitro* diagnostics. One of the main activities of SPIDIA is to improve whole blood processing. In this study, a magnetic bead robotic system, the QIAsymphony® SP, was used to develop a fully automated purification of RNA[1] and concomitant enrichment of miRNA from whole blood collected in PAXgene® Blood RNA Tubes[2,3]. More than 100 chemistries and protocol conditions were screened resulting in an optimized method for maximizing miRNA yield from stabilized whole blood. Data are presented which demonstrate the performance of this optimized protocol in comparison to five other isolation methods.

Figure 1. A: BioRobot MDx, B: QIAsymphony

The applications presented here are for research use only. Not for use in diagnostic procedures.

Material and methods

Blood was collected into PAXgene Blood RNA Tubes from consented healthy adults, stored for 20-24 h at room temperature and frozen at -20°C. After 2h thawing they were subjected to RNA isolation by the QIAsymphony (optimized) extraction protocol and five other RNA extraction procedures, three of them dedicated to miRNA isolation (table 1).
A Spectramax photometer and an Agilent Bioanalyzer were used to determine quantity and quality of the resulting RNA. MiRNA yields were determined by quantitative RT-PCR using commercially available SYBR green or probe based (QIAGEN or Life Technologies) assays on Rotorgene Q (QIAGEN) or BioMark (Fluidigm) instruments. Due to the small reaction volume of 9 nanoliters on the BioMark arrays we performed a pre-amplification of the cDNA using 14 cycles with 1:100 diluted primer set that was used in the following detection assays.

Table 1. Overview of compared methods

Kit	Protocol	Technology	miRNA	Method	Abbreviation	Ref.
*QIAsymphony PAXgene Blood RNA**	*QIAsymphony*	*silica beads*	*no*	*large RNAs*	*QS*	*1*
mod. QIAsymphony PAXgene Blood RNA	QIAsymphony opt.	silica beads	yes	co-isolation	QS miRNA	---
PAXgene Blood RNA MDx	BioRobot MDx cust. 1	silica memb.	yes	co-isolation	MDx miRNA 1	5
PAXgene Blood RNA MDx	BioRobot MDx	silica memb.	no	large RNAs	MDx	5
mod. PAXgene Blood RNA MDx	BioRobot MDx cust. 2	silica memb.	yes	small RNAs only	MDx miRNA 2	---
PAXgene Blood miRNA	manual	silica memb.	yes	co-isolation	miRNA man	4
PAXgene Blood RNA	manual	silica memb.	no	large RNAs	RNA man	2

* basis of QS miRNA but not tested in this study

Results – Yield and purity

Total RNA yields were > 3µg for all samples and the yield minimum specification was met for all PAXgene Blood Kits (≥ 3µg for 95% of all samples with a WBC of 4.8-11 x 10^6/ml).

Average yield of the newly developed method QS miRNA was 9.1µg.

Average yields of automated samples preparation methods were slightly lower compared to their manual counterparts.

Average purity (260nm/280nm) of all methods was in the range between 1.8 and 2.2. with exception of method MDx miRNA 2 (1.4).

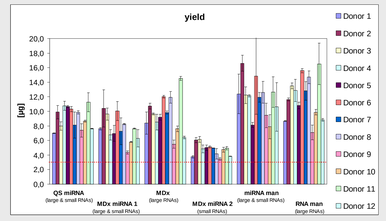

Figure 2. Total yields achieved with all tested methods. Dotted red line shows the minimum specification of all PAXgene Blood RNA kits.

Results – Agilent bioanalyser

Analyzing samples with Agilent Bioanalyser Nano or Pico LabChips resulted in high RIN values for all used methods (table 2) that (co-)purify large RNA species.

The high amount of small RNAs purified with the optimized QIAsymphony PAXgene Blood RNA Kit and protocol had a slightly negative impact on RIN values (figure 3). This shows that the RIN (and the algorithm behind it) is probably not the method of choice to quality control RNA eluates containing small RNAs (including miRNAs).

Table 2. RNA integrity

Kit	Method	Abbreviation	mean RIN
mod. QIAsymphony PAXgene Blood RNA	co-isolation	QS miRNA	**8.0**
PAXgene Blood RNA MDx	co-isolation	MDx miRNA 1	**8.0**
PAXgene Blood RNA MDx	large RNAs	MDx	**7.9**
mod. PAXgene Blood RNA MDx	small RNAs only	MDx miRNA 2	---
PAXgene Blood miRNA	co-isolation	miRNA man	**7.5**
PAXgene Blood RNA	large RNAs	RNA man	**8.3**

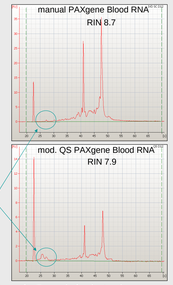

Figure 3. Exemplaric electropherograms

Results – SYBR Green based Assays

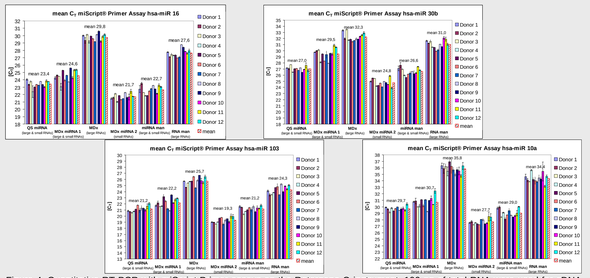

Figure 4. Quantitative RT-PCR with miScript Primer assays on the Rotorgene Q instrument. 100 ng of total RNA were used for cDNA synthesis. Resulting cDNA was diluted 1/50 or 1/5 depending on the specific miRNA expression in blood. For protocol MDx miRNA 2 the amount of template was calculated by combining yields of MDx and MDx miRNA2.

Results – Probe based assays

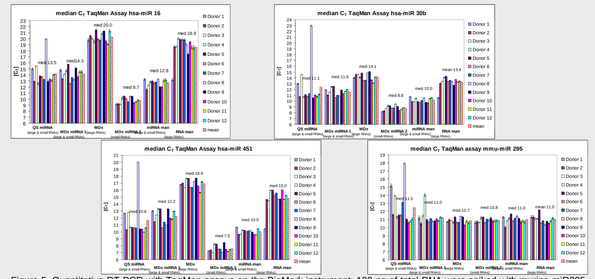

Figure 5. Quantitative RT-PCR with TaqMan assays on the BioMark instrument. 100 ng of total RNA were spiked with murine miR295 prior to cDNA synthesis. Resulting cDNA was pre-amplified and diluted 1:4 prior to expression analysis on a 96.96 Dynamic Expression array. For protocol MDx miRNA 2 the amount of template was calculated by combining yields of MDx and MDx miRNA2.

Conclusions

➢ We developed the first whole blood sample processing chemistry and protocol that uses a magnetic bead based, automated system for the isolation of small RNAs, especially miRNAs.

➢ Agilent Bioanalyser RIN values are probably not the best tool to judge the integrity of small RNA containing samples.

➢ Eluates generated with this method gave high yields and good purities. They are ready for use in downstream applications like quantitative RT-PCR.

➢ We demonstrated a clear enrichment of miRNAs similar to membrane based methods.

References

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About SPIDIA

The SPIDIA consortium has been formed by merging the complementary expertise of the different consortium partners. SPIDIA involves 16 partners from 11 different European countries. It consists of 7 private research companies, 8 public research organizations (universities, hospitals & biobanks), along with the European Standards Organisation, CEN.
It has adapted overall critical mass of budgeted 1567 person-months. All partners are European world-class leaders in their fields of activity, publishing in the best and inventing key products for the medical and scientific community. Furthermore, the consortium has been extended by a Club of Interest in order to have an optimal dissemination of the obtained results.
The SPIDIA project is coordinated by QIAGEN GmbH, Germany, the world's leading provider in sample and assay technologies for life science, applied testing, pharma, and molecular diagnostics.
Partner AROS is the leading provider of Total Genomic Solutions in Europe. Partner PreAnalytiX develops, manufactures and sells integrated and standardized systems for collection, stabilization and purification of nucelic acids from blood, bone marrow and tissue specimens.

Table 4. SPIDIA Partners

Name of Organization	Country
Medical University of Graz	Austria
European Committee for Standardisation	Belgium
Biotechnology Institute of the Czech Academy of Sciences	Czech Republic
Aros Applied Biotechnology A/S	Denmark
Dako Denmark A/S	Denmark
ACIES	France
ImmunID Technologies	France
QIAGEN	Germany
Technical University Munich	Germany
Consorzio Interuniversitario di Risonanze Magnetiche di Metalloproteine Paramagnetiche	Italy
University of Florence	Italy
Istituto Nazionale dei Tumori Fondazione IRCCS	Italy
Erasmus Medical Center Rotterdam	Netherlands
DIAGENIC ASA	Norway
TATAA BIOCENTER AB	Sweden
PreAnalytiX GmbH	Switzerland

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