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BIOBANKING FOR GLOBAL CHALLENGES

Transcriptomics in 17 year-old blood samples? Yes, we can!

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INTRODUCTION

Analysis of blood RNA transcription profiles is hindered by *ex vivo* gene expression changes, as well as degradation and loss of RNA over time during specimen transportation and storage.

The PAXgene Blood RNA System was developed by PreAnalytiX to overcome these problems. So far, quality control data has shown that the PAXgene Blood RNA System stabilizes and preserves RNA in blood specimens for up to 11 years of storage at -20°C and -70°C.

To enable transcriptomic analyses, the Norwegian Women And Cancer (NOWAC) Post-genome Cohort chose the PAXgene Blood RNA Tubes for a nation-wide blood sampling in 2003.

AIM

With this work, our aim was to answer the question: could the 17 year old NOWAC blood specimens still be used for research?

METHOD

We analyzed 198 blood specimens from the NOWAC Post-genome cohort. Specimen collection took place in 2003 at family doctor's offices form all over Norway, and specimens were shipped by mail at uncontrolled conditions to the UiT Core Facility for Biobanking in Tromsø. Here, the specimens were frozen and kept at -80ºC for 17 years until shipment on dry ice to PreAnalytiX/QIAGEN lab in Hilden, Germany.

Specimens were thawed and equilibrated to room temperature. Isolation of RNA was done using the PAXgene Blood RNA Kit on the QIAcube instrument according to the protocol of the kit handbook.

We analyzed RNA quantity and purity (UV spectroscopy, qPCR), integrity (Agilent Bioanalyzer) and suitability for gene expression analysis (RT-PCR inhibition assay). Samples with traces of gDNA in qPCR were subject to DNase I treatment and RNA clean-up before re-analysis.

All methods applied were thoroughly validated according to USP <1225> (1) and ICH Q2 (R1) (2), to prove through series of studies that they met the intended analytical applications. All instruments and devices used were regularly maintained and used according to SOPs.

RESULTS

From a total of 198 blood specimens, 2 were lost during sample preparation, due to user error. 10.7% of all specimens did not produce a visible pellet after PAXgene Blood RNA Tube centrifugation, although sample processing turned out successful later, as shown with acceptable RNA yields.

Samples qualified by low gDNA contamination (≤1% gDNA/total NA w/w for 149 of 196 samples) showed highly intact RNA of RIN 8.7 (median), ranging from RIN 6.9 to 9.6 (Fig. 1).

RNA yield was 6.6µg/2.5mL blood (median), with 96.0% of samples (143/149) showing ≥2.5µg RNA /2.5mL blood (Fig. 2.A). The lowest RNA yield was obtained from a PAXgene Blood RNA Tube which was heavily underfilled with blood (0.5µg RNA/tube, sample no. 123).

For 99.3% of samples (148/149), RNA purity A_{260}/A_{280} was between 2.0 and 2.2 with median purity of 2.09 (Fig. 2.B). One sample did not meet the general criterion of A_{260}/A_{280} between 1.8 to 2.2 (sample no. 123).

All RNA samples were subjected to qPCR of ACTB to determine the amount of gDNA. 96.9% of all samples (190/196) showed minimal contamination of the RNA by less than 1.0% gDNA of total nucleic acids w/w (Fig. 3.A), with median of 0.33% gDNA w/w (Fig. 3.B).

None of the RNA samples showed inhibition of a downstream RT-PCR assay, determined by assaying two different amounts of template RNA per sample (Fig. 4).

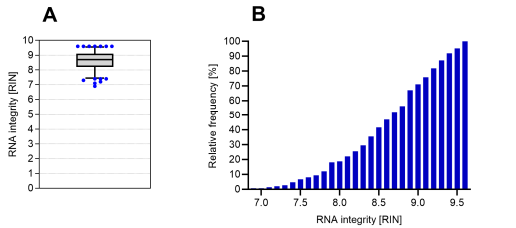

Fig. 1: RNA integrity analysis by microcapillary gel electrophoresis (n=149). (A) Box and whiskers plot of RIN numbers, with median (center line), 25th to 75th percentiles (box), 5 and 95 percentiles (whiskers), and outliers (dots). (B) Frequency distribution of RIN values.

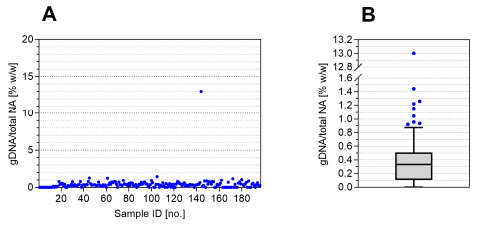

Fig. 3: Detection of gDNA by RT-PCR of ACTB (n=196). (A) Relative amount of gDNA (% gDNA w/w per total nucleic acids of sample). (B) Distribution of gDNA contamination. Box and whiskers plot with median (center line), 25th to 75th percentiles (box), 5 and 95 percentiles (whiskers), and outliers (dots).

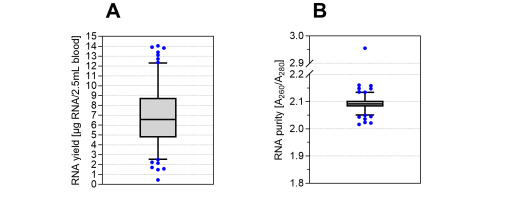

Fig. 2: RNA yield and purity analysis by UV spectroscopy (n=149). (A) Box and whiskers plot of RNA yield, with median (6.6 µg per 2.5mL blood, center line), 25th to 75th percentiles (box), 5 and 95 percentiles (whiskers), and outliers (dots). (B) Box and whiskers plot of RNA purity ($A_{260}/_{280}$), with median (2.09, center line), 25th to 75th percentiles (box), 5 and 95 percentiles (whiskers), and outliers (dots).

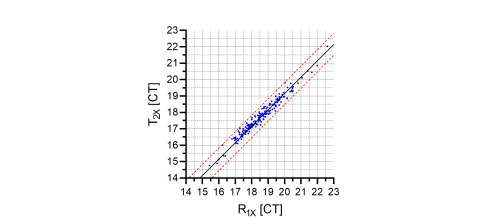

Fig. 4: Real-time IL1B RT-PCR inhibition assay results (n= 196). The scatter plot shows the CT values of reference and test RT-PCRs with 1 and 2 volumes of template RNA input (R1x, T2x) per RNA sample. The solid black line indicates the expected correlation of CT values, and the red lines show the upper and lower limit of methodological variation (ΔCT +/- 3σ with ΔCT = CT[R1x] – CT[T2x] and σ = total precision of the assay).

CONCLUSIONS

RNA extraction from all specimens yielded sufficient amounts of highly pure, low gDNA contaminated and highly intact RNA, suitable for use in RNA downstream assays without any inhibition.

The de-centralized NOWAC blood specimen collection procedure and shipping of specimens at ambient temperatures is likely to have introduced variability in RNA yield, due to lack of standardization of these important pre-analytical steps.

When used according to ISO 20186-1 (3), the system helps to standardize the steps for venous whole blood cellular RNA examination in what is referred to as the pre-examination phase as part of the entire workflow from specimen collection to the cellular RNA examination.

We conclude that the PAXgene Blood RNA System provides stabilized, high quality RNA from blood specimens stored for up to 17 years at -80°C.

REFERENCES

1) United States Pharmacopeia, General Information / <1226> Verification of Compendial Procedures

2) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Validation of Analytical Procedures: Text and Methodology

3) ISO 20186-1 Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 1: Isolated cellular RNA

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